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A New Genus and Species of Dermanyssidae (Acarina: Mesostigmata) from the English Sparrow, with Observations on its Life Cycle*

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Bird-mites of the family Dermanyssidae have recently been the subject of epidemiological studies in connection with the spread of the American encephalitides, but, as of late, the nature of their role in the spread of these diseases has been questioned (Chamberlain and Sikes, 1955, Reeves *et al.*, 1955, Sulkin *et al.*, 1955). Recently a new mite was discovered which falls into this category (*i.e.*, dermanyssid; parasitic on birds).

In May, 1955, a juvenile English sparrow, *Passer domesticus domesticus* (Linné, 1758), covered with mites, was brought into the laboratory by Mr. Lee Roper. The bird was placed in a bird-mite culture receptacle of the type used by Chamberlain and Sikes (1950) and some specimens were mounted in Hoyer's medium for determination. When microscopic examination revealed that they were members of a new genus and species of the family Dermanyssidae, the nest from which the bird had fallen was recovered and placed in culture, using a week-old chick as host.

Pellonyssus, n. gen.

Dorsal shield of females divided into two subequal halves, the posterior one slightly smaller; male dorsal shield entire; covering most of unengorged idiosoma in both sexes. Males with entire holoventral shield; sternal shield of females reduced. First pair of sternal setae reduced; metasternal setae absent; a fourth pair of pores present on minute platelets in both sexes. Epigynial shield of females pointed posteriorly, with one pair of setae. Anal plate oval; cribrum punctate; anus in anterior region of plate, with paired adanal setae arising at posterior level of anal opening. Coxae without ventral spurs. Chelicerae with widened base, the remainder long and attenuate; chelae shearlike but reduced, both fixed and movable digits present. With the following stages in life cycle: egg, larva, protonymph, deutonymph and adult. Parasites of birds.

TYPE: *Pellonyssus passeri* new species.

The generic name *Pellonyssus* is derived from two Greek words, the archaic πέλλα meaning skin or hide and νύσσω meaning to prick.

Mites of the genus Pellonyssus superficially resemble those of *Steatonyssus*. This resemblance disappears, however, when one compares the ventral surfaces of the two genera. The genus *Steatonyssus* was erected by Kolenati in

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1858, and the type was subsequently designated as *Acarus musculi* Schrank, 1803. In his diagnosis of this genus, Fonseca (1948) listed a large dorsal podosomal shield and a well developed, elongate, dorsal opisthosomal shield in the female; an entire dorsal shield in the male; a sternal shield in the female with three pairs of setae and two pairs of pores; the genital plate of females tapering to a point, with one pair of setae; and an entire holoventral shield in males. Typical *Steatonyssus* spp. have, in addition, normal sternal and metasternal setae as well as a large sternal shield which is heavily chitinized on the posterior edge; while *Pellonyssus* has reduced first sternal setae, no metasternal setae, and a reduced sternal shield which is not heavily sclerotized. The chelicerae and chelae of the two genera also differ markedly. In *Pellonyssus* they are reduced, appearing intermediate in form between those of *Dermanyssus* Duges, 1934 and those of *Ornithonyssus* Sambon, 1923 (=*Bdellonyssus* Fonseca, 1941), while *Steatonyssus* has large, well developed chelicerae and chelae. Host preferences indicate that *Steatonyssus* spp. are found on mammals, particularly bats, and that *Pellonyssus* is a bird parasite. Zumpt and Till (1954) in their key to *Steatonyssus* include four species from birds. However, all of these show characters of *Pellonyssus* and are herein referred to this genus. They are the following new combinations: *Pellonyssus viator* (Hirst, 1921), *P. biscutatus* (Hirst, 1921), *P. reedi* (Zumpt and Patterson, 1952), and *P. similis* (Zumpt and Till, 1954).

Pellonyssus passeri, n. sp. (Plates I-III)

ADULT FEMALE (Pl. I) Body approximately 427 microns wide at peritremes, 688 microns long exclusive of gnathosoma, light brown to bright red to dark brown, depending upon engorgement.

Venter. (Pl. I, fig. 1) Tritosternum with two lacinae, vaguely pilose. Sternal shield reduced, crescentic, about 145 microns wide, 14 microns long. First pair of sternal setae as long as anterio-posterior length of sternal shield; second pair of sternal setae three times as long as first pair; third pair of sternal setae six times as long as first pair. Two pairs of pores on sternal shield; third pair of pores on idiosoma near genital opening; fourth pair on small platelets, on margin of epigynial shield. Metasternal setae absent. Epigynial shield pointed posteriorly, reaching past fourth coxae, with one pair of setae. Anal plate egg-shaped; anal opening in anterior part of plate, with paired adanal setae arising at posterior level of anus. Single postanal seta smaller than adanals and arising anterior to beginning of cribrum on anal plate. Stigmata situated near anterior level of coxa IV; peritreme in plate, extending anteriad and slightly dorsad, terminating at posterior level of coxa II. Narrow endopodal plates coalescing with peritremal plate. Fifteen or sixteen pairs of opisthosomal setae situated from level of genital setae to posterior of body, their position varying with engorgement. Idiosomal platelets minute.

Dorsum. (Pl. I, fig. 2) Dorsal shield divided, covering most of idiosoma in unengorged specimens. Anterior shield about 275 microns wide at posterior end, 296 microns long, roughly in the shape of an equilateral triangle, reticulate at margins and with ten pairs of setae: one vertical, five lateral and four median. Posterior shield slightly narrower than base of anterior shield, about 213 microns wide at anterior end, and 323 microns long; anterior margin concave at midline; shield tapering to plunt point, strongly reticulate, with six pairs of setae, all marginal or submarginal. Thirty-one to thirty-two pairs of setae on idiosoma, exclusive of shield setae.

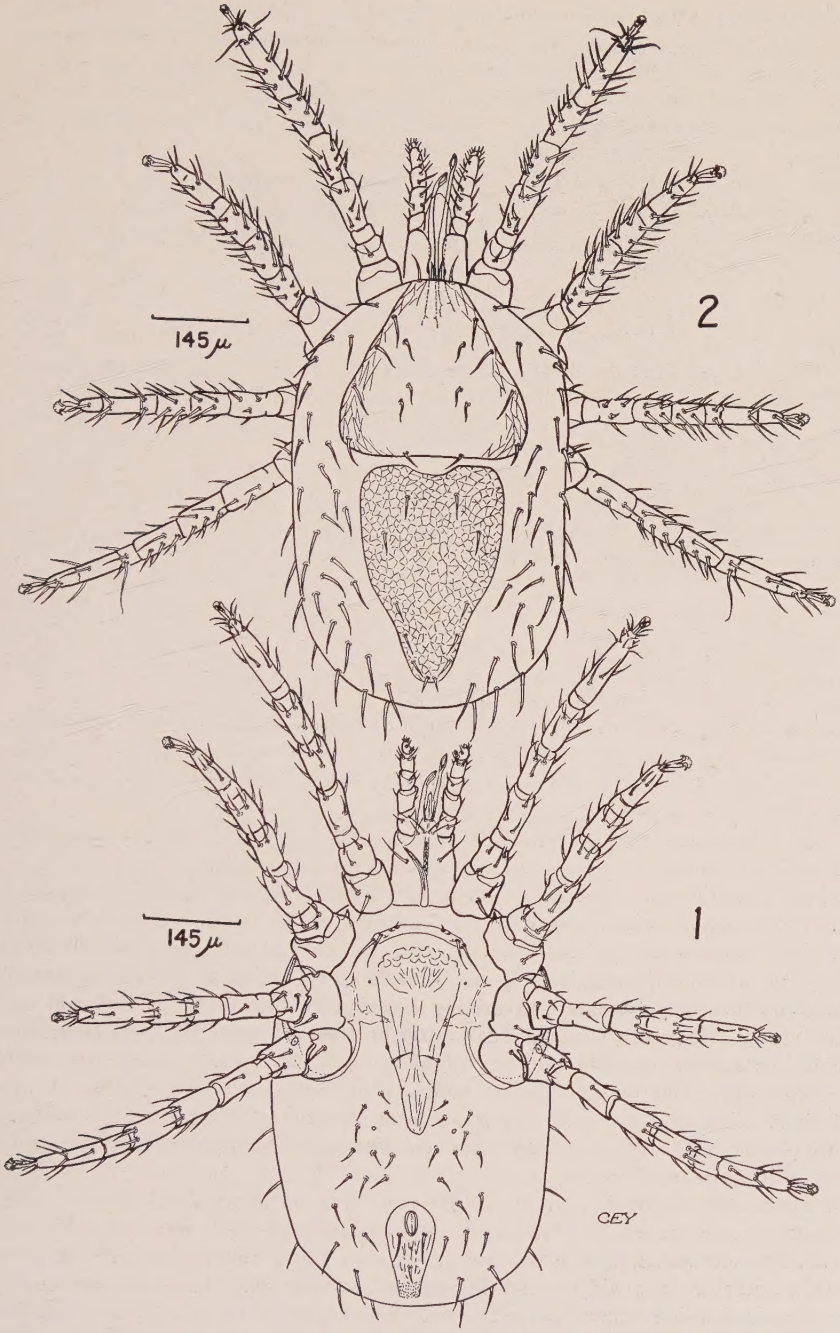

PLATE I

Pellonyssus passeri n. sp. (female)

Fig. 1 = venter Fig. 2 = dorsum

All figures drawn with the aid of a microprojector

Gnathosoma. Palpal segments devoid of spines; deutosternal teeth ten in number, arranged consecutively along deutosternum. Cheliceral base widened, 24 microns wide, 15 microns long; remainder of chelicerae long and attenuate, 7-10 microns wide, 220 microns long, terminating in shearlike chelae. Chelae reduced, but both fingers present, similar to those of *Ornithonyssus bursa*.

Legs. All legs with claws and caruncle; length of legs: I = 537 microns, II = 461 microns, III = 463 microns, IV = 585 microns; chaetotaxy typically dermanyssid. Coxa II with anterio-dorsal spur sharply pointed; other coxae without spurs.

MALE. (Pl. II, figs. 1, 2, and 5) Body approximately 315 microns wide at peritremes, 570 microns long, exclusive of gnathosoma. Color as in female. Holoventral plate faintly reticulate, tapering posteriorly with a shallow constriction in the region of coxa IV. Three pairs of sternal setae, the anterior pair reduced; metasternal setae absent; one pair of genital setae opposite coxae IV. Paired adanal setae situated at level of mid-region of anal opening; unpaired seta just anterior to finely punctate cribrum. Three additional pairs of setae present between genital and paired anal setae. Four pairs of pores present on podosomal region of plate, the fourth pair on small platelets just in front of the genital setae opposite coxa IV. Unsclerotized opisthosomal venter with seven paired setae. Stigmata between coxae III and IV. Peritremal plate curving regularly; length, from posterior edge of stigmal opening to anterior end of sclerotized plate, approximately 123 microns. Tritosternum as in female. Anterio-dorsal spur present on coxa I; other coxae lacking spurs; legs typically dermanyssid.

Dorsal plate entire, tapering regularly to a blunt point, 256 microns wide, 513 microns long, covering about three-fourths of unengorged body, generally with 15 pairs of setae and six pores. Approximately 21 pairs of setae on unsclerotized dorsum.

Chelicerae shorter and stouter than in female, basal segment expanded, remainder elongate and narrow. Length of apical segment, including chelae, about 145 microns. Spermatodactyl approximately 38 microns in length, relatively stout, tapering externally to a narrow curved trough, a distinct subapical tooth occurring internally. Fixed chela elongate, narrow, with reduced sclerotization, an elongate duct-like depression occurring at the apex. Eleven deutosternal teeth are present.

DEUTONYMPH. (Pl. II, figs. 3 and 4) Body approximately 342 microns wide at peritremes, 445 microns long, exclusive of gnathosoma. Generally more weakly sclerotized than protonymph or adult, with finer setae. Color bright red to orange red as in freshly fed protonymph or adult. Tritosternum well developed, with a broad base which splits, approximately 26 microns distally, into two smooth, slender arms about 72 microns in length. Sternal plate weakly sclerotized and indistinct. Three pairs of short sternal setae present; one pair of genital setae opposite coxae IV. Anal plate broad, weakly sclerotized, with a seta on either side of the anal opening. Unpaired seta anterior to weakly punctate cribrum. About nine paired setae on unsclerotized opisthosomal venter. Peritremal plate 84 microns from posterior edge of stigmal opening to apex of sclerotized plate. Coxa II with a short, anterio-dorsal spur; other coxae without spurs.

Dorsal plate weakly sclerotized and poorly defined. Dorsum with about 30 pairs of small setae.

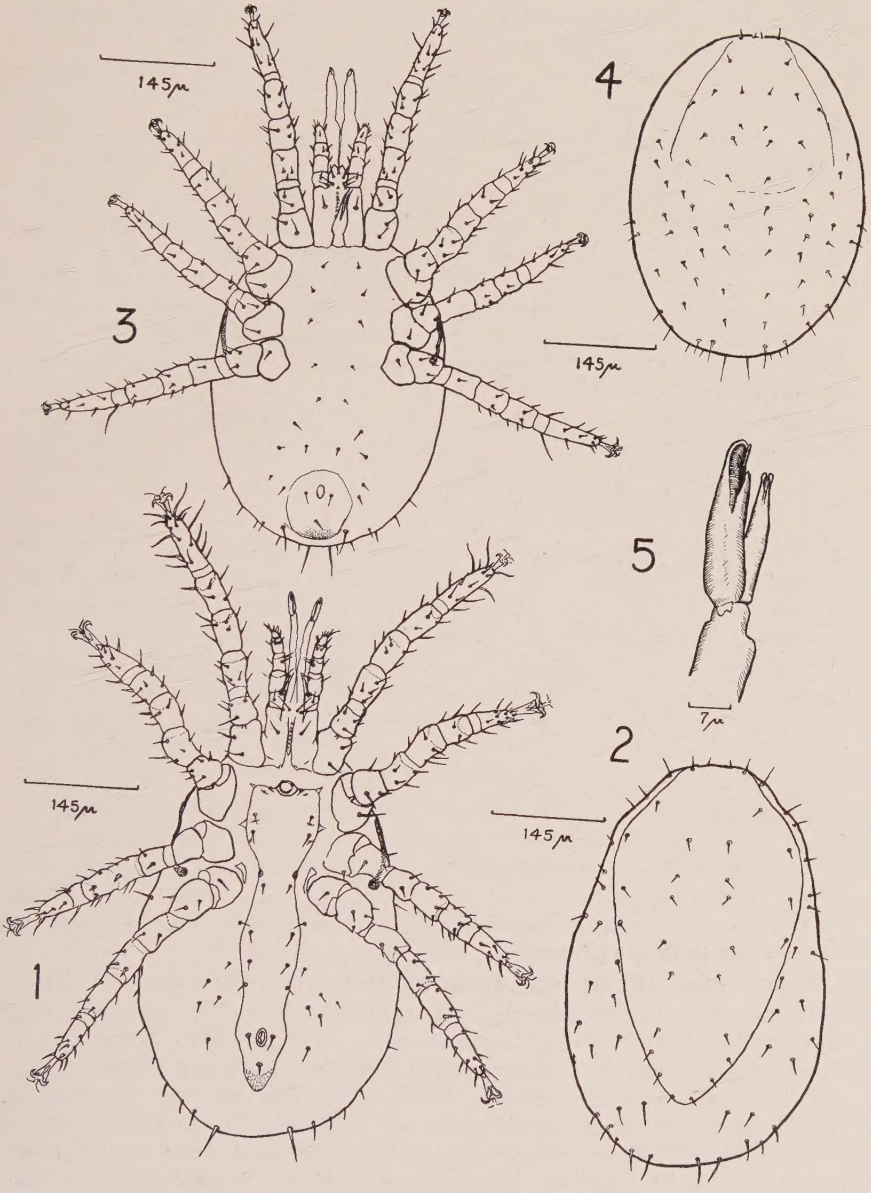

PLATE II

Pellonyssus passeri n. sp. (male and deutonymph)

Fig. 1 = male, venter
Fig. 2 = male, dorsum
Fig. 3 = deutonymph, venter
Fig. 4 = deutonymph, dorsum
Fig. 5 = male chela

All figures drawn with the aid of a microprojector

Chelicerae reduced, non-functional and lightly sclerotized. Length of apical segment including chela 63 microns; bud-like chelae about eight microns long. Palps with few setae and relatively immovable. Nine deutosternal teeth arranged consecutively along deutosternum.

PROTONYMPH. (Pl. III, figs. 1 and 2) Body approximately 342 microns wide at peritremes, 553 microns long, exclusive of gnathosoma. (This stage varies considerably in size from the unfed to mature condition.) Sternal plate distinct, 75 microns wide, 106 microns long, tapering to a blunt point, with three pairs of equal setae and two pairs of pores. A pair of pores in integument opposite coxae IV. Metasternal and genital setae lacking. Anal plate 50 microns wide, 65 microns long; anus near anterior margin. Three anal setae present, the unpaired considerably shorter than the paired. Five pairs of setae on unsclerotized opisthosoma. Peritremal plates 35 microns long, slightly curved, without a stigmal opening. Coxa II with small, spiniform anterio-dorsal spur; other coxae without spurs. Tritosternum elongate, lacinae vaguely pilose.

Dorsal plates four: a large anterior plate with eight pairs of equal setae, a pair of small irregular plates on anterior opisthosoma, and a pygidial plate on the posterior opisthosoma with two pairs of setae. Unsclerotized dorsum with 15 pairs of setae.

Chelicerae functional, elongate, slender and flexible. Chelae small and shear-like. Apical segment of chelicerae 141 microns long, including chelae. Deutosternal teeth seven in number.

LARVA. (Pl. III, figs. 3 and 4) Body approximately 200 microns wide at coxae IV, 311 microns long, exclusive of gnathosoma. Whitish in color, with reduced sclerotization and setation. Sternal plate vague and indefinite, with three pairs of small setae. Single pair of setae on uncovered opisthosomal venter. Anal plate poorly defined, with three setae. Tritosternum distinct, lacinae not pilose, 14 microns wide at base, 70 microns long.

Dorsum lightly sclerotized, without distinct plates. Dorsum lacking setae except at posterior end where three pairs of long, whip-like setae occur.

Chelicerae reduced, non-functional, apical segment, including bud-like chelae, 70 microns long.

EGG. Egg a translucent, pearly oval, approximately 228 microns wide, 314 microns long. Shell without ornamentation, covered with a clear, sticky substance with binds egg to substrate. Embryo discernable within maturing egg.

TYPE SPECIMENS. The holotype female USNM #22182218 is deposited in the collection of the United States National Museum, Washington, D. C. Paratype specimens, representing all stages described are deposited in the collections of the United States National Museum and the Institute of Acarology, University of Maryland, College Park, Md. In addition, paratype adult specimens will be deposited in the following institutions: The Chicago Academy of Sciences; The Chicago Natural History Museum; The British Museum (Natural History), London, England; Muséum National d'Histoire Naturelle, Paris, France; Institut Royal des Sciences Naturelles de Belgique, Bruxelles, Belgium; Universitetets Zoologiske Museum, Copenhagen, Denmark; Rijksmuseum van Natuurlijke Histoire, Leiden, Holland; Riksmuseum, Stockholm, Sweden; Natal Museum, Pietermaritzburg, South Africa; and the South Australian Museum, Adelaide, South Australia. All type material was selected from a single culture of a sparrow's nest.

TYPE LOCALITY. A sparrow's nest, College Park, Maryland inside of University of Maryland armory.

TYPE HOST. *Passer domesticus domesticus*, the English sparrow.

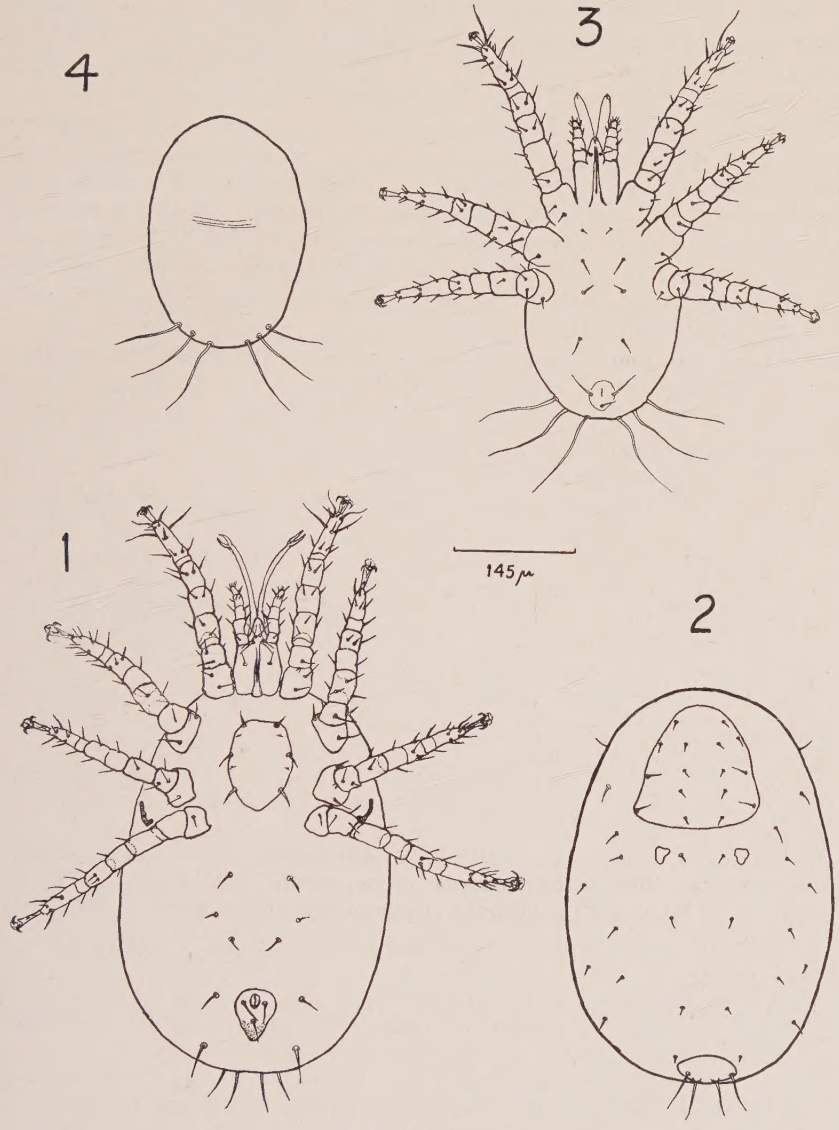

PLATE III

Pellonyssus passeri n. sp. (protonymph and larva)

Fig. 1 = protonymph, venter
Fig. 2 = protonymph, dorsum
Fig. 3 = larva, venter
Fig. 4 = larva, dorsum

All figures drawn with the aid of a microprojector

Diagnosis. *Pellonyssus passeri* may be distinguished from all other species of *Pellonyssus* by the following characters or combination of characters: female anterior dorsal shield with a straight posterior border; setae at posterior of opisthosoma not greatly enlarged over ventral body setae; peritremal plate elongate, running from anterior level of coxa II, curving beneath coxa IV and coalescing with endopodal plates; sternal shield ten times as wide as long. Protonymph with two small dorsal platelets in addition to large anterior dorsal plate and smaller pygidial plate.

Biology. Observations on this species, both in culture and on the host, indicates that it appears similar, in general, to members of the genus *Ornithonyssus*. The eggs are laid in protected areas of the environment and have not been found on the host. The egg hatches shortly into a sluggish, hexapod larva whose chelicerae are not developed for feeding. The larva molts into the protonymphal stage, which is an active feeder, and may be found running over the nest or host with considerable speed. Upon maturing the protonymph molts to the deutonymph which, like the larva, is an inactive form without functional chelicerae. The deutonymph remains quiescent in a protected spot until, with a final moult, the adult condition is attained. Since the deutonymphal and larval stages are relatively short and are passed in a sheltered environment, these forms are not recovered as readily as are the protonymphal and adult stages.

This species was successfully cultured for 15 days on a young chick in the laboratory. At the end of this time, it was necessary to terminate the culture due to its contamination with acarid mites.

Remarks. *Pellonyssus passeri* is the eighth species of Dermanyssidae to be recorded from the English sparrow. Previously *Dermanyssus gallinae* (DeGeer, 1778) has been reported on *Passer domesticus* in North America by Ewing (1911, 1922); *D. longipes* Berlese and Trouessart, 1889 in Great Britain by Hirst (1916); *D. americanus* Ewing, 1922 in North America by Ewing (1922) and Miles *et al.* (1951); *D. passerinus* Berlese and Trouessart, 1889 in North America by Hoyle (1938) and in Great Britain by Hirst (1922). *Ornithonyssus sylviarum* (Canestrini and Fanzago, 1877) has been reported from *Passer domesticus* in North America by Rayner (1932) and in South America by Fonseca (1948); *O. bursa* (Berlese, 1888) in Great Britain by Hirst (1916), in South America by Fonseca (1948) and in Hawaii by Alicata (1947); *O. canadensis* (Banks, 1905) in North America by Ewing (1922, 1947); and *O. iheringi* (Fonseca, 1935) in South America by Fonseca (1948). Specimens of *Pellonyssus passeri* were also collected from *Passer domesticus* in Beltsville, Md., during 1954 by Mr. G. I. Wilson (personal communication).

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SUMMARY

1. A new genus and species of Dermanyssidae are described, in all stages, from the English sparrow, *Passer domesticus domesticus* (Linné, 1758).

2. Systematic characters are given which distinguish the new genus from *Steatonyssus;* and the generic diagnosis of *Steatonyssus* is amplified.

3. Four species of bird-mites, previously included in the genus *Steatonyssus,* are referred to the new genus.

4. Some observations on the biology, in culture, of the new species are included.

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A Digestion Method for Post-mortem Recovery of Nematodes from Ruminants

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Post-mortem determination of the helminth population of cattle is commonly based on an estimate of the number of worms recovered from the gastrointestinal tract and other organs or organ systems (Porter, 1942). A determination so made does not account for the specimens which may be embedded in tissues. In the case of the stomach worms, particularly *Ostertagia ostertagi,* large numbers may be missed.

Bailey and Herlich (1953) recovered 24,000 immature and 30,000 mature *O. ostertagi* from the abomasal contents of a mature bovine. They observed moreover, that the mucosal surface of the abomasum bore numerous nodules typical of infection with this parasite. Blendorization and digestion of a 30-gram piece of abomasal mucosa resulted in the recovery of 2,000 additional immature *O. ostertagi,* suggesting that from about 30,000 to about 50,000 such worms were embedded in the mucosa of the entire organ. Moreover, Sommerville (1953) observed that *O. circumcincta,* a parasite of sheep closely related to *O. ostertagi,* had a rather definite distribution in the abomasum, and observations at this laboratory suggest that *O. ostertagi* also has a definite predilection for the folds of the fundus gland region of the abomasum. Consequently, any estimate based on worms recovered from an excised piece of tissue would of necessity vary considerably with the particular site chosen for excision.

It follows from the above considerations that a technic is needed in which the entire abomasum can be so treated that all or nearly all the embedded nematodes in it can be recovered. Blendorization of an entire abomasum, particularly from an adult bovine, is an unwieldy job, requiring that this organ be cut in numerous, small, manageable pieces. Such a procedure has the additional disadvantage of strands of tissue, especially of the serosa, becoming entwined about the shaft of the blendor.

The pepsin-hydrochloric acid digestion technic (Schwartz, 1939) has long been employed for the liberation of trichinae larvae from the musculature. Sprent (1952) used a modification of this technic for the recovery of ascarid larvae from the tissues of white mice.

In the course of post-mortem examinations, the author of this paper thoroughly washed the abomasa of five calves and three lambs, experimentally infected as shown in table 1, and then subjected each abomasum to digestion without prior cutting or grinding. The digestion solution was prepared as outlined by Schwartz (1939), namely, 600 ml. water, 10 ml. concentrated HCl, and 5 to 6 gms. pepsin for each half-pound of abomasum to be digested. The material was placed in a graduated glass container and left overnight in an incubator kept at 37° C. In every case the entire mucosa was completely digested the next day, and only strands of serosa remained undigested in most instances. An aliquot of the digested material was collected and examined for nematodes directly under the stereoscopic microscope.

Table 1.—Worms recovered by washing and digesting abomasa of experimentally infected calves and lambs

Animal Number*	No. and kind of larvae fed	No. Worms Recovered		Percent of worms recovered by digestion to total number recovered
		Contents and Washings	Digested Abomasa	
C 1	300,000 *O. o.***	11,000	1,560	14.1
C 2	500,000 *O. o.*	180,000	49,840	27.7
C 3	200,000 *T. a.****	18,180	16,460	47.5
C 4	500,000 *T. a.*	66,200	12,000	18.1
C 5	250,000 *O. o.*	210,000	4,220	2.0
	250,000 *T. a.*	147,000	6,380	4.3
L 1	50,000 *T. a.*	4,580	3,120	40.5
L 2	25,000 *T. a.*	4,540	11,450	71.7
L 3	25,000 *T. a.*	940	6,050	85.3
	25,000 *O. o.*	460	200	13.8

*C — calf, L — lamb
**_Ostertagia ostertagi_
***_Trichostrongylus axei_

Table 1 summarizes the results of digesting five calf and three lamb abomasa in the above-described manner. The data show that nearly 50,000 worms would have been unaccounted for in calf 2 had not this technic been used and in calf 3 nearly half of the *Trichostrongylus axei* would have been missed.

Fourth- and fifth-stage *T. axei,* as well as sexually mature adults and *O. ostertagi* in every stage of development have been recovered from digested abomasa. Almost every specimen so recovered was in satisfactory condition for identification, and in many instances living worms were recovered.

Up to the present, the intestinal tract of only one naturally infected calf has been subjected to digestion, and 6.9 percent of all *Cooperia punctata* specimens were recovered from the digested material. In addition, fourth-stage specimens of *Oesophagostomum radiatum* were recovered from the digest of the ileum and cecum.

SUMMARY

A procedure utilizing the hydrochloric acid-pepsin digestion technic is described for the recovery of nematodes embedded in the tissues of the gastrointestinal tract of ruminants. This procedure led to the recovery of many nematodes which would not have been found, had the usual post-mortem technic been used to collect parasites from the gastrointestinal tract.

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Experimental Infections of Guinea Pigs With *Trichostrongylus colubriformis*, a Parasite of Ruminants

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Investigations on parasites of ruminants are of necessity limited by the relatively great expense entailed in maintenance of the host animals. It would be desirable, therefore, to conduct studies on these parasites in small animals in order to obtain information of a fundamental nature on host-parasite relationships as well as on treatment to expel these pests.

Zavadovskii and Zakharova (1931) recovered specimens of *Trichostrongylus axei* (=*T. extenuatus*) and *T. colubriformis* (=*T. instabilis*) from one of nine guinea pigs fed infective larvae cultured from the feces of a roe deer. They observed eggs being passed with the feces 25 days after infection of the guinea pig, and at post-mortem examination, four days later, they recovered three male and eight female worms. One male was *T. axei* and the other two were *T. colubriformis*. Ortlepp (1939) infected rabbits with *T. colubriformis* of sheep origin and then infected sheep with larvae of this same parasite, cultured from fecal pellets from the infected rabbits. Roth (1941) reported the recovery of one male *T. colubriformis* from a guinea pig, and surmised that this host had become infected accidentally by ingesting a larva with fresh grass cut from an area grazed by sheep.

We conducted two trials of experimental infections of guinea pigs with *T. colubriformis*, and the results are herewith presented.

In the first trial, each of eight guinea pigs was fed 1,500 infective larvae of *T. colubriformis* cultured from the feces of an experimentally infected calf. The guinea pigs were killed 1, 2, 3, 4, 5, 6, 10, and 27 days, respectively, after infection, and immature worms were recovered from the small intestines of all except the last-killed animal. The worms so recovered were slightly more advanced in their development than were worms recovered from calves at comparable periods after infection, as determined by one of us (FWD) in a study of the life history of *T. colubriformis* in calves.

The number of worms present post-mortem was not determined for the guinea pigs killed 1, 2, and 3 days after infection. In the remaining five animals, however, the number of worms recovered and percent of infective dose that these numbers represent were 98 (6.5%), 450 (30%), 100 (6.7%), 1,110 (74%), and 651 (43.4%).

The feces examined daily from the one guinea pig kept alive 27 days became positive for *Trichostrongylus* eggs 16 days after infection, and eggs were still passing in the feces 11 days later, when the animal was killed. The egg count ranged from an initial 50 EPG (eggs per gram of feces) to a peak of 3,602, four days later. The count then dropped rapidly until, at time of necropsy it was reduced to 194 EPG. Of 651 mature worms recovered from this guinea pig, only 23 were still in the small intestine. The remaining ones were in the cecum and colon as far down as the rectum, indicating that the worms were passing out and the infection was terminating. The worms recovered were identified as *T. colubriformis*.

Table 1. Source and number of *Trichostrongylus colubriformis* larvae used to infect six guinea pigs and subsequent egg count data

Guinea Pig Number	Weight* (gms.)	Larvae Fed		Prepatent Period (days)	Maximum EPG	
		Number	Source		No.	Day
9	280	2,000	C**	19	2	19
10	315	500	C	18	26	22
11	274	2,000	G.P.***	15	334	18
12	264	500	C	15	16	19
13	821	2,000	C	18	16	21
14	817	2,000	G.P.	15	570	18

*Weight at time of administration of infective larvae
**C — larvae of calf origin
***G.P. — larvae of guinea pig origin

Fecal pellets were collected from the aforementioned guinea pigs and cultured on sphagnum moss or charcoal. Infective larvae from these cultures were used to infect two guinea pigs in the second series of trials. At the same time four other guinea pigs were fed infective larvae cultured from a calf. Larvae of calf origin were 10 days older than the larvae of guinea pig origin at the time of administration.

As shown in table 1, the fecal egg counts of the two guinea pigs infected with larvae of guinea pig origin were much higher than the counts recorded for the guinea pigs infected with larvae of calf origin, probably indicating a larger number of worms in the first two animals. It is not known whether this apparent difference in worm number was due to a rapid adaptation of this parasite to the guinea pig following one passage through this host, or to the difference in age of the infective larvae at time of their administration, or to some other intrinsic factor, such as individual host response. The peak egg count, with the exception of guinea pig No. 9, was observed on the third or fourth day after the initial appearance of eggs. Four guinea pigs were still passing eggs 33 days after infection, the other two having become negative in 27 days.

The results of these trials indicate that the guinea pig can apparently serve as an excellent experimental animal for studies of the nematode, *T. colubriformis,* since a relatively high percentage of the infective larvae fed succeed in reaching sexual maturity. The worms produced viable eggs which were passed with the host's feces, and the eggs could be cultured to obtain larvae which, in turn, were infective to parasite-free guinea pigs.

SUMMARY

Two series of experiments are reported in which 14 guinea pigs were successfully infected with *T. colubriformis,* a nematode parasite of ruminants.

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A Study of Watercress in Hawaii as a Possible Source of Human Infection with Liver Flukes *(Fasciola)**

Joseph E. Alicata** and David D. Bonnet***

Flukes, *Fasciola gigantica,* are commonly found as parasites in the liver of cattle in the Hawaiian Islands. Wild pigs are also known to be infected (Shipley, 1913), but the incidence among them is not known. In Hawaii these flukes utilize the lymnaeid snail *Fossaria ollula* as intermediate host (Alicata, 1938). Cattle usually become infected as a result of eating vegetation growing in wet areas and containing encysted metacercariae. These parasites ordinarily perpetuate among herbivorous animals, but man can also become infected by ingesting the encysted immature flukes. During the past fifty years, there have been observed in the Hawaiian Islands at least 19 cases of human fascioliasis (Alicata, 1953; Stemmermann, 1953). The first cases were reported by Herbert in 1907, and the latest one, which proved to be fatal, occurred in 1951 (Alicata, 1953). Most of these human infections have been found by physicians during surgical operations or in other accidental ways. This seems to indicate that other cases most likely have occurred, but have remained unrecognized.

Although the source of liver fluke infection in man is unknown, it can be safely assumed that the encysted metacercariae must have been ingested from eating infected uncooked vegetation grown in water, or from drinking water containing the encysted forms. In this connection, the late Dr. G. Herbert in 1906, and the late Dr. M. C. Hall in 1936, suggested that the common habit of eating raw watercress in the Islands was probably the main source of human infection. Because of the fact that liver flukes are widespread locally among cattle and possibly wild pigs, and because the snail vector is ubiquitous in fresh water, the possibility of infection does exist if watercress is irrigated with water contaminated with excreta of infected animals.

This report deals with a study carried out during 1953 to determine if local watercress sold commercially is predisposed to conditions conducive to fluke cysts infestation, and the desirability of setting up an adequate control program.

Methods of Investigation

In this study a visit was made to all commercial watercress growing farms in the Hawaiian Islands. In each area visited, the following observations and collections were made: (1) topography of the area, source of water used for irrigation, and likelihood of the water becoming contaminated with feces of cattle or wild pigs; (2) presence of cattle in or nearby the area; whenever possible, fecal samples from these animals were collected and examined for liver fluke eggs; (3) presence and abundance of lymnaeid snails; when present, representative samples were collected and examined for various developmental stages of liver flukes.

Following the above observations, an attempt was made to give a parasi-

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tological evaluation of each farm examined. The terms "satisfactory," "unsatisfactory," and "doubtful" were chosen. The word "satisfactory" was used for an area where under normal conditions there appeared to be no likelihood for the water supply used to become polluted with feces from cattle or wild pigs. The term "unsatisfactory" was used for an area where liver fluke infection was found among the snails collected, and conditions were such that it would have been uneconomical for the grower to improve conditions. The word "doubtful" was used for an area where there was possibility of fecal pollution from cattle or wild pigs and no liver fluke infection was found in the snails collected; in most of these places some adjustment needed to be made by the grower to improve conditions. Under this designation it was also implied that there is a lack of basic information on the role which wild pigs play locally in the dissemination of fluke infection in nature.

RESULTS AND DISCUSSION

A summary of this study is presented in table 1. Thirty-three commercial watercress-growing farms in four of the larger islands of the Territory were surveyed. The number in each island was as follows: Hawaii, 9; Oahu, 13; Maui, 6; and Kauai, 5. Of the 33 farms, 12 (36 percent) were evaluated as "satisfactory," 1 (3 percent) as "unsatisfactory," and 26 (61 percent) as "doubtful." The farm on the island of Hawaii which is recorded as unsatisfactory used water from a stream originating uplands where cattle were known to be present. About 7 percent of the lymnaeid snails collected from this farm were found infected with various developmental stages of liver flukes. The identity of these flukes was ascertained from experimental infection of guinea pigs and rabbits with encysted cercariae which had been derived from the snails.

Of the 20 farms which were designated as "doubtful," one or more of the following conditions were found: (a) in two farms, it appeared possible for surface water from adjacent pastures to flow in the watercress area; (b) in ten farms, there appeared the possibility of contamination from adjacent swamps in which cattle had been grazing; (c) six of the farms were receiving water originating from uplands in which wild pigs were believed to be present; (d) in five farms, cattle were allowed to graze in or nearby the water-

Table 1. Summary of Results of the Watercress Farms Surveyed in the Hawaiian Islands

	Hawaii	Oahu	Maui	Kauai	Total
Areas surveyed	9	13	6	5	33
Areas considered as "satisfactory"	4	5	1	2	12
Areas considered as "unsatisfactory"	1	0	0	0	1
Areas considered as "doubtful"	4	8	5	3	20
Areas in which lymnaeid snails were found	9	10	6	2	27
Areas showing possible contamination of surface water from adjacent cattle grazing pasture	1	0	1	0	2
Areas showing possible contamination from adjacent swamp	0	6	1	3	10
Areas using stream water originating from uplands	1	1	2	2	6
Areas showing cattle grazing nearby	0	4	1	0	5
Areas in which spring water became contaminated with stream water	2	0	1	1	4

cress plots. In four of these places fecal samples of the cattle examined showed evidence of liver fluke infection; (e) in four of the farms, stream water originating from uplands was allowed to mix with the spring water used in raising watercress.

Of the 33 watercress farms examined, 27 (82 percent) showed presence of lymnaeid snails. Of these, the following abundance of snails were recorded on the various islands: Hawaii, 4 with many, 1 with a moderate number of, and 4 with few snails; Oahu, 3 with many, and 7 with few snails; Maui, 2 with many, 3 with moderate, and 1 with few snails; Kauai, 1 with moderate, and 1 with few snails.

The information gathered in the present survey shows that in several of the farms studied, the potentiality did exist for infection of the watercress with the encysted liver fluke. Following this study, a discussion was had with the various watercress growers and the following suggestions, aimed at the control of watercress infection, were made:

1. Use spring water or known clean water, as the source of water supply and raise the watercress in the vicinity of the spring outlet.
2. Avoid using stream water as a source of water supply. Such water may be contaminated from infected cattle or wild pigs grazing upstream.
3. Avoid nearby surface ground water from emptying into the watercress area. When necessary, ditches should be dug along the sides of the watercress area to prevent such contamination.
4. Do not allow cattle to graze in or nearby watercress areas as the water may become contaminated with the droppings of these animals.
5. Avoid growing watercress in swampy land or immediately next to swamps.
6. Possible use of a molluscacide, such as copper sulphate, for the control of the snails in the watercress area.

Summary

Thirty-three commercial watercress farms in the Hawaiian Islands were visited to ascertain possible presence of conditions which may influence infection of the watercress with liver fluke (*Fasciola*) cysts. An attempt was made to classify each farm as "satisfactory" if no likelihood of infection existed, "unsatisfactory" if fluke infection was found in the lymnaeid snails collected in the area, and "doubtful" if possibility of infection appeared to exist.

Of the 33 farms surveyed, 12 were evaluated as satisfactory, 1 unsatisfactory, and 20 as doubtful. Control measures aimed at the prevention of fluke infection in the watercress have been suggested.

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Rotylenchus christiei, n. sp., a New Spiral Nematode Species Associated with Roots of Turf

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The genus *Rotylenchus* Filipjev, 1934, contains several species of nematodes all of which are undoubtedly plant parasites. They feed upon their hosts for the most part ectoparasitically, sometimes lying partially embedded in the plant tissue, though instances are known where they become completely embedded. These organisms are probably world-wide in distribution in temperate and tropical countries where they attack a large variety of plants, including various crops and ornamental plants, and produce obvious damage. Organisms of this genus, as well as those of *Helicotylenchus* Steiner 1945, are commonly known as spiral nematodes because of the spiral shape generally taken what at rest or dead.

The species described here was obtained from turf in Florida where it was presumably feeding on the roots of the grasses. The material was collected by Mrs. A. J. Overman of the Gulf Coast Experiment Station, Bradenton, and sent to Dr. J. R. Christie of the University of Florida, who in turn, kindly brought the specimens to the authors. *Rotylenchus christiei*, n. sp. is of particularly interest because morphologically it differs so greatly from other forms of the spiral nematode group.

MEASUREMENTS: 12 ♀. Length, 0.801 mm. (0.751 mm. to 0.860 mm.); a, 24.8; b, 6.0; c, 60.2; V,[28] 56.[26]
12 ♂. Length, 0.710 mm. (0.630 mm. to 0.789 mm); a, 26.3; b, 5.2; c, 35.1.

FEMALE: Body cylindrical for most of its length, with the anterior one-fifth tapering to the rounded lip region which at its base is about one-third as wide as the widest part of the body near the vulva (Fig. 1A). Lip region not distinctly set off by a constriction, probably with 3 striae, though these are very poorly developed and have been seen only on occasional specimens. The tail is shorter than the anal body diameter and very bluntly convex conoid, the exact shape varying slightly, but usually asymmetrical (Fig. 1, B). The cuticle is marked by annules of nearly uniform width. On the lateral fields there are two plainly visible lines beginning shortly behind the anterior end and extending to near the terminus. On most of the body, the transverse striations approach but do not quite touch these lines, leaving a narrow strip of unmarked cuticle (Fig. 1, B). On occasional specimens, particularly larvae and young females, two additional longitudinal lines located outside the plainly visible ones can be seen, at least on parts of the body. In lateral view, the hemizonid is seen as a very slight irregularity in the second annule anterior to the excretory pore. Deirids were not seen. Externally, the phasmids are seen as oval structures occupying the entire space between the 2 visible lateral lines (Fig. 1, D). These are always located in the same general region of the body (Fig. 1, A), but never opposite each other, either right or left phasmid being anterior with about equal frequency. In 12 specimens of each sex measured, the anterior phasmid was located at an average of 79% of the body length (maximum 83%, minimum 77%), while the posterior phasmid was located at an average of 86% of the body length (minimum 84%, maximum 90%). Average distance between the phasmids

was 50 microns (minimum 39, maximum 62).

The stylet in 12 specimens had an average length of 32 microns (minimum 30, maximum 33) and was of the form characteristic of the genus (Fig. 1, C). The opening of the dorsal oesophageal gland is about 5 microns posterior to the base of the stylet.

The median bulb of the oesophagus is about half as wide as the body at that point. Posterior to the median bulb is a short isthmus surrounded by the nerve ring. The oesophageal glands form a lobe overlapping the anterior end of the intestine. The exact form of this lobe varies considerably, being elongated in some specimens and so shortened in others as to nearly resemble a bulb. This situation is much like that described by Thorne (1949) for *R. robustus*. The cells of the intestine are filled with globules of varying size; details obscure. The rectum is short and often obscure.

The transverse vulva is located a little posterior to the middle of the body and in all specimens observed had protruding lips as shown (Fig. 1A). These apparently consist of a cuticular flap on each lip of the vulva, extending the full width of the latter. From the vulva a vagina with a well developed cuticular lining extends inward a little less than half the body width. From it one uterus extends anteriorly and one posteriorly, both ending in well developed seminal receptacles. The two ovaries are outstretched and have a single line of oocytes except in a short region of multiplication.

Male: The anterior portion of the male body is much like that of the female, while the tail is surrounded by a well developed bursa (Fig. 1F). Phasmids (Fig. 1E), as well as the lateral fields and annulation, are as described for the female. The stylet is slightly shorter than that of the female, averaging 29 microns (maximum 30, minimum 28).

The single outstretched testis ends near the middle of the body and has a double row of cells in the germinal zone, about 6 rows of cells in the growth zone, and a seminal vesicle containing apparently globular sperms. The curved spicules of 12 males had an average length of 30 microns (maximum 31, minimum 28). The slightly curved gubernaculum is a little more than one-third as long as the spicules.

Diagnosis: *Rotylenchus* differing from other known species of the genus in the presence of protruding lips of the vulva, in the forward location of the male and female phasmids and the fact that they are not opposite each other on specimens of either sex, and in the presence of only two easily visible lines instead of four on the lateral fields of most mature specimens.

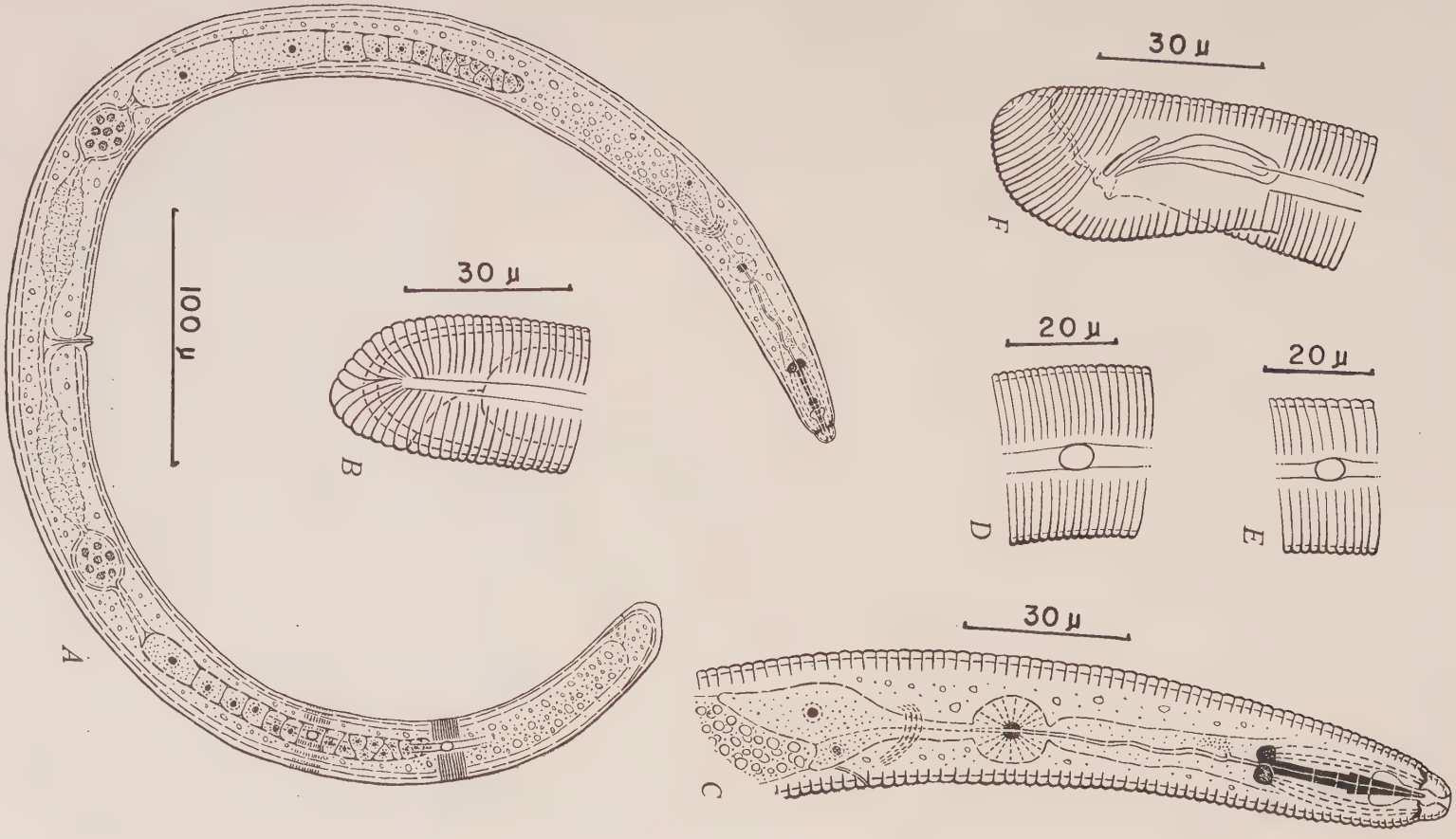
Holotype—Female; Slide No. 101, Collection of Section of Nematology, Beltsville, Maryland.

Allotype—Male; Slide No. 102, Collection of Section of Nematology, Beltsville, Maryland.

Paratypes—Males and females; Collection of Section of Nematology, Beltsville, Maryland; Mr. Gerald Thorne, Section of Nematology, Salt Lake City, Utah; and University of California Nematode Survey Collection, Berkeley, California.

Type habitat and locality—Soil around roots of Bermuda grass (*Cynodon dactylon* (L.) Pers.) and "spotted spurge weed" (Euphorbiaceae) on 14th fairway of the Bobby Jones Municipal Golf Course, Sarasota, Fla.

Fig. 1. *Rotylenchus christiei*, n. sp. A—Female. B—Tail end of female. C—Anterior portion of female. D—Phasmid, lateral field and annulation of female. E—Phasmid, lateral field and annulation of male. F—Tail end of male.


100 µ
A
30 µ
B
30 µ
F
20 µ
D
20 µ
E
30 µ
C

The species name is given in honor of Dr. J. R. Christie of the University of Florida.

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Studies on Resistance to the Root-knot Nematode of the Genus *Meloidogyne* Goeldi, 1887.

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In a paper published in 1953, the writer (Edwards 1953) recorded for the first time in the Gold Coast the presence of a root-knot nematode of the genus *Meloidogyne* Goeldi, 1887. It was made known that it had been found on eleven species of cultivated plants and on three species of indigenous wild hosts. A further contribution two years later (Edwards 1955) revealed that altogether seventy-six host plants, both cultivated and wild, were subject to attack in West Africa and that these various plant species showed marked differences in the reaction of their root tissues to invasions. In the course of these later and more intensified studies, some species of cultivated plants were found totally resistant to invasion of their roots by the parasite while in the case of others the degree of resistance was less complete, the larvae being able to enter the root tissues and even, in some plant species, develop to the adult stage and give rise to small numbers of ova despite the stunting influence of the host.

The systematic relationship of the root-knot nematode present in the Gold Coast has not been definitely established but it must be considered to belong to a hitherto undescribed species distinguished from the six species and two subspecies already described in the genus Meloidogyne (Chitwood, 1949; Chitwood, Specht and Havis, 1952; Loos, 1953) largely on differences in perineal pattern and other, less constant, morphological features. The description of this new species with a detailed account of the anatomy of the adult and larval forms will be the subject of a separate communication by the writer. It may be added that the results of tests designed for identification on the basis of host reactions (Sasser, 1952) points to the conclusion that this new species is closely related to *M. incognita* (Kofoid and White, 1919) Chitwood, 1949. Groundnuts (peanuts) are not attacked while watermelons, wheat and other cereals as well as pepper constitute highly susceptible host plants to infestation.

Methods

The present paper embodies the results of extensive tests carried out under field conditions at the Government Farm, Pokoasi, Gold Coast, during 1955, when the cultivated plants which had shown resistance to the root-knot eelworm, judging from the results of general field observations made in previous years, were grown on land heavily infested by the parasite. In addition, a few other plant species were included since no information was available concerning their susceptibility to attack by this particular nematode in the Gold Coast.

The entire area selected for these tests, after being cleaned thoroughly of all weeds and cultivating well to an appreciable depth, was ridged, the distance between the ridges being 36 inches. All the plant species included in the tests were grown on top of the ridges, a single row on each ridge. The ground was retained free of all weeds from the time the plants were sown until they reached full maturity. Throughout this period of growth, the plants themselves were kept under constant observation for any adverse effects in their aerial parts due to the root-knot nematode having invaded the root system. At weekly intervals, entire plants of each species complete with their roots, were also carefully lifted for detailed examination in the laboratories at the University College of the Gold Coast. Finally, on each occasion, a representative sample of the roots of each plant species was stained with acid fuschin in lacto-phenol (Goodey 1937) for detection of the parasite within the root tissues.

RESULTS

***Arachis hypogaea* Linn. (Groundnuts).** Two varieties of *Arachis hypogaea* Linn. commonly grown in West Africa were tested for their resistance to attack by the root-knot nematode, namely, var. Natal Common and var. Kumawu Erect. Despite very exhaustive search made of the roots of these two varieties not only in the course of the present investigations but also on numerous occasions in previous years in several localities in the Gold Coast, no specimens of any phase in the life cycle of the parasite has ever been recorded within the root tissues of either variety. Hitherto, there has been no opportunity to examine other varieties of this plant growing on infested land in West Africa.

***Crotalaria* species.** Six different species of Crotalaria were included in the trials, *C. juncea* Willd., *C. anagyroides* H. B. et K., *C. striata* Schum. et Thonn., *C. usaramoensis* E. G. Baker, *C. retusa* Linn. and *C. spectabilis* Roth. As expected from the results of general field observations made in previous years, all these species proved highly resistant to the root-knot eelworm present in the Gold Coast. The degree of resistance varied in the different species. It is evident that the parasite can support itself on the roots of *C. juncea* and reproduce in their tissues but at a very low rate of multiplication. The invasion leads to gall formation but the galls produced are few in number and small in size, being only just visible to the naked eye. Each gall seldom contains more than one fully matured female and although ova and young larvae may be present, it is somewhat doubtful, judging from the results of observations hitherto made, that such larvae are able to penetrate deeper into the tissues and give rise to new foci of infection.

The larvae of this parasite are also able to enter the rootlets of *C. anagyroides, C. striata* and *C. usaramoensis,* and develop, at least a small proportion of them, to the adult stage. It is only on very rare occasions that ova are produced or any visible nodules formed due to the presence of the young, developing worms in the root tissues. The species *C. retusa* and *C. spectabilis* are totally resistant to the root-knot nematode, at least that species present in the Gold Coast. It is noteworthy in this respect that the *C. retusa* included in these tests is the local, indigenous form, and that on one occasion in the case of plants grown in another locality in the Gold Coast from imported seed of this species, slight swelling of the roots due to the presence of larvae of the parasite was observed by the writer. None of the larvae developed, however, to the adult stage.

***Gossypium hirsutum* Linn. and *G. barbadense* Linn. (Cotton).** Two varieties of cotton, Marie Galante and Ishan, belonging to *Gossypium hirsutum* Linn. and *G. barbadense* Linn., respectively, were investigated as to their susceptibility to attack by the parasite. Both are perennial species but *G. barbadense* can be treated as an annual. Two other varieties of cotton labelled D28 and A9215 were also tested. The roots of all plants examined in the four varieties of cotton remained throughout the season free of infection by any species of nematodes except for the variety Marie Galante. Appreciable numbers of *Pratylenchus* sp. in all stages of development from egg to adult occurred in its roots and, among them, a few young larvae of the root-knot eelworm were noted on occasions. No other stage in the life cycle of the root-knot nematode was detected in this variety at any time. The variety Marie Galante tested is the indigenous form commonly found growing spontaneously on the Accra Plains in the coastal belt of the Gold Coast.

***Oryza sativa* Linn. (Rice).** Increasing areas of rice, *Oryza sativa* Linn., both paddy (swamp) and upland (dry land) varieties, are being grown annually in West Africa. The paddy variety of rice had been cultivated for several years on a narrow strip of land separating the experimental ground from the edge of a lake or reservoir. The upland variety was sown alongside the paddy variety and, in common with it, no nematodes of any species were discerned at any time within its root-tissues.

***Trifolium resupinatum* Linn. (Persian clover).** Seed of various species of plants are imported from time to time into the Gold Coast to test their value, under West African conditions, as cover crops on arable land for three specific purposes, the regeneration of soil fertility, the suppression of weeds, and the provision of herbage for green-manuring or fodder for the feeding of farm animals. Seed of *Trifolium resupinatum* Linn. had been received for these purposes but some of it was sown to ascertain the behavior of this plant when grown on land heavily infested with the root-knot nematode. On one occasion, a single specimen of an immature female of this parasite was seen in a rootlet but despite further exhaustive examinations made at the time and on subsequent occasions during the season, no confirmation that this plant species is subject to attack was obtained. The possibility that the root with the immature female within its tissues constituted part of the root system of another plant species harbouring the nematodes cannot be definitely ruled out in this particular case.

***Centrosema pubescens* Benth.** *Centrosema pubescens* Benth. constitutes one of the plant species introduced in recent years into the Gold Coast to find out its potentialities as a herbage plant for forming a thick, dense sward and, simultaneously, providing useful food for cattle and sheep. It quickly established itself and, indeed, soon developed in many districts into a serious menace, spreading at an alarming rate over wide areas and often crowding out more valuable crops. Judging from the results obtained in the present tests on its resistance to the root-knot nematode, it can be regarded as highly immune to this parasite. It was only on exceptionally rare occasions that any specimens of the parasite were observed within its root and none of them had developed beyond the final larval stage.

***Medicago sativa* Linn. (Lucerne).** The germination of the seed of *Medicago sativa* Linn. proved extremely poor and only ten plants established themselves and these never appeared at any time luxuriantly healthy. The special search made of the entire root system of each individual plant lifted

at the rate of one specimen every two weeks over a period of about five months failed to reveal the presence of any stage of the root-knot eelworm within the root tissues. These results taken by themselves indicate that *M. sativa* is not attractive to this parasite, at least in the Gold Coast, but definite conclusions, however, cannot be reached until more comprehensive tests with this plant are conducted.

***Glycine hispida* Maxim. (Soya bean).** General field observations made by the writer in various parts of West Africa had indicated that certain varieties of soya bean seldom show violent root reactions when grown on land harbouring the root-knot nematode. Consequently, it was decided to test eleven varieties of this plant species for their comparative degree of resistance to the parasite, namely, vars. Acadian, Avoyelles, Benares, Black Forage, Ex Rayon, Fort Lamy, Java Forage, Lassa No. 4, White Biloxi, No. 459 and 28 E.B. The roots of all varieties were soon invaded by the larvae of the root-knot eelworm and within thirty days of germination they contained irrespective of the variety, adult female worms with egg-masses. Six weeks after germination there were no visible galls on the roots of var. Fort Lamy and those present in vars. Acadian, Avoyelles and Lassa No. 4, were few in number and barely visible to the unaided eye. At this stage of growth, White Biloxi, 28 E.B. and Benares showed the most serious root reactions and they were the only varieties with compound or multiple galls on their roots.

The final examination was carried out twelve weeks after germination when most of the flowers had set and produced good pods. The variety Acadian was still remarkably free of galls but the variety Lassa No. 4 had failed to retain the superiority displayed by it in this respect earlier in the season. The varieties Fort Lamy and Avoyelles could not be regarded as seriously attacked, judging by the number and size of the galls on their roots but in the case of the varieties Java Forage and Black Forage, some of their roots had become distorted, twisted swollen structures, virtually valueless to the plant. Contrary to expectations, based on the results of examinations made in early stages of growth, the variety White Biloxi was relatively free of serious root manifestations and in this respect it compared fairly favourably with the varieties Fort Lamy and Avoyelles. In general, the remaining varieties were intermediate between these three types and the forms Java Forage and Black Forage.

It would seem from the results obtained in this investigation that soya beans are able to withstand attacks and remain comparatively free of serious root distortion due to the root-knot nematode, at least in the Gold Coast. The extent of the root malformation, however, varies in the different varieties.

***Pueraria thunbergiana* Benth. (Tropical Kudzu).** In the Gold Coast, *Pueraria thunbergiana* Benth. rapidly establishes itself when sown on arable land and produces, except in the arid regions, such a dense growth of luxurious foliage throughout the year that all species of weeds are completely kept in check. Such a plant species capable of smothering all weeds, if also immune to attack by the root-knot nematode, would prove exceedingly valuable for the control of this parasite in tropical soils. There had been no evidence that *P. Thunbergiana* suffers from the ravages of any soil-inhabiting pests but when it was grown in the present investigation on land severely infested by the root-knot eelworm, its roots developed manifestations characteristic of serious attack by this parasite. Despite the high populations of the nematode in the roots and the marked gall formation, none of the plants

displayed any adverse effects in their aerial parts.

***Symphytum peregrinum* Ledeb. (Russian Comfrey).** A consignment of crown sets of *Symphytum peregrinum* Ledeb. arrived by air in the Gold Coast soon after the field tests on the immunity of selected plant species to infection by the root-knot eelworm had been started. This was the first importation of *S. peregrinum* into the Gold Coast and, although this plant species was introduced largely to find out its performance as a fodder crop under West African conditions, it was also tested for its susceptibility to depredations of the root-knot nematode. It soon became evident that the plant is highly attractive to this eelworm and that its roots quickly develop into massive deformed structures under the influence of secretions of the parasite. Although this abnormal development did not lead to immediate crisis in the metabolism of the plants, the enormous yield of succulent foliage normally claimed for this plant species was never produced.

***Zea mays* Linn. (Maize).** Maize, *Zea mays* Linn. is extensively grown over the greater part of the Gold Coast except in the dry savannah regions of the north and the dry Accra plains along the coast in the south. It constitutes the most favoured crop for land under arable cultivation and three or four crops are successfully grown each year in soils of satisfactory moisture content. The importance of crop rotation is not generally appreciated by growers in the Gold Coast with the result that the land is often cropped repeatedly with the same plant species until its fertility is virtually exhausted or its pest content assumes a serious proportion. In the case of continuous cropping with maize, however, a serious build up of infestation of the soil by the root-knot nematode has not been witnessed anywhere.

Two varieties of maize, Tsolo and American White, were included in the present tests for resistance to this parasite. Neither variety proved highly attractive to the eelworm but occasionally a few galls were encountered near the tips of some of the young developing roots. The galls were globular to oval in shape and small in size, being normally only just visible to the unaided eye and each containing only a single female worm. Almost invariably in all cases examined, the adult female was situated so near to the periphery of the gall that the vulva and the gelatinous mass of ova around it were exposed on the outside of the root. There was also evidence which pointed to the conclusion that as the plants draw towards maturity and rapid growth of their tissues ceases, they are more vulnerable to a slightly heavier infection and gall formation.

It can be definitely stated that, under the conditions in which these trials were conducted, both varieties of maize, Tsolo and American White, are very resistant indeed to attack by the root-knot nematode and that of the two varieties, Tsolo is slightly superior in this respect.

***Sorghum vulgare* Pers. (Guinea corn).** Guinea corn, *Sorghum vulgare* Pers., is another species of Gramineae extensively grown on arable land in the Gold Coast but, in contrast to maize, largely in the savannah areas of the northern region where reductions in yields of crops due to the presence of the root-knot eelworm are unknown. Compared with the results obtained in the case of the two varieties of *Zea mays* in the present studies, it is evident that *S. vulgare* is more attractive to the nematode or at least offers less resistance to the parasite establishing itself and reproducing within its root tissues. The difference in this connection between the two plant species is not appreciable.

***Phaseolus lunatus* Linn. (Lima bean).** It is frequently claimed by Africans and Europeans who are keen gardeners that the Lima bean, *Phaseolus lunatus* Linn., is the most successful pulse crop for cultivation in the Gold Coast for human consumption and that it does not suffer even if sown on land known to harbour the root-knot eelworm. In the present trials, it was grown, along with all the other plant species tested, on land heavily infested by this parasite. It was found that even under these conditions it appeared to grow normally, making an extraordinarily rapid, dense growth, and commencing within about six weeks to produce pods large enough for human consumption.

Although the nematode enters and multiplies freely in the roots of the Lima bean, its presence seldom results in violent reactions in the form of large compound galls and malformation of the root system. In general, the galls are comparatively abundant and small in size, being normally only just visible to the naked eye and with the vulva of the female worm and its egg-mass protruding through the epidermis to the outside of the root. It would seem that auto-infection is, as in the Gramineae, uncommon in the Lima bean, that is, infection from larvae having hatched within the root and migrated to an adjacent fresh tissue within the same root to start a new focus of infection. In general, as in plant species of the family Gramineae, new foci of infection are started by invading larvae which have hatched in the soil or on the external surface of the root system of plants.

It is evident that there is a state of equilibrium between the Lima bean and the root-knot eelworm or at least it is able to withstand attacks and remain relatively free of root malformation. This may be due, at least in part, to the exceptionally rapid growth made by the plant.

***Stizolobium deeringianum* Bort (Velvet bean).** The Velvet bean, *Stizolobium deeringianum* Bort., in common with the Lima bean, proved itself a prolific grower and capable of producing well developed pods within seven weeks of germination, though the ground in which it was growing carried a heavy infestation of the root-knot nematode. It displayed great resistance to this eelworm but occasionally a few fully-matured females with ova were discerned in some of its young developing roots, particularly in the earlier stages of growth of the plants. There was little or no indication of gall formation.

***Cicer arietinum* Linn. (Chick pea), *Lathyrus sativus* Linn. (Bitter vetch), *Vicia villosa* Roth. (Hairy vetch), *Solanum tuberosum* Linn. (Potato), *Urena lobata* Linn. and *Leucaena glauca* Benth.** These six plant species were included in the present tests since they were either new introductions into the Gold Coast or no information whatsoever was available as to their susceptibility to attack by the parasite or parasites in West Africa. Chick pea, *Cicer arietinum* Linn., Bitter vetch, *Lathyrus sativus* Linn., and Hairy vetch, *Vicia villosa* Roth., proved highly suitable for multiplication by the root-knot nematode and liable to develop extreme root manifestations characteristic of serious attacks by this eelworm. *Urena lobata* Linn. and potato, *Solanum tuberosum* Linn. (var. Majestic), also proved suitable for the maintenance and reproduction of the parasite but the galls present on the roots were comparatively small and did not result in marked root deformation. The deep rooted plants of *Leucaena glauca* Benth. remained remarkably free of infection, judging by the very low number and the small size of the galls on the roots as well as by the relatively few female worms which succeeded to reach maturity.

Note on Rotylenchulus Reniformis

In the course of these studies, nematodes of *Rotylenchulus reniformis* Linford and Oliveira, 1940, were encountered on the roots of:

Cicer arietinum Linn. Chick Pea
Crotalaria anagyroides H.B. et K.
Crotalaria striata Schum. et Thonn.
Phaseolus lunatus Linn. Lima Bean
Pueraria thunbergiana Benth. Tropical Kudzu
Solanum tuberosum Linn. Potato
Stizolobium deeringianum Bort. Velvet Bean
Vicia villosa Roth. Hairy Vetch
Zea mays Linn. Maize, American White

The intensity of root infestation, except in the case of *Crotalaria* spp. and *Pueraria thunbergiana,* was exceedingly light. Although the nematodes have been classified as coming in the species *Rotylenchulus reniformis* it must be pointed out that there are morphological differences which may warrant on further investigation the creation of a separate species for them.

Grateful acknowledgements are due to Mr. W. Smith, Senior Agricultural Officer, Ministry of Agriculture of the Gold Coast, for his most valuable co-operation in connection with the field operations involved in these studies.

Summary

The root-knot nematode of the genus *Meloidogyne* was recorded for the first time in the Gold Coast, West Africa in 1953. It has been the subject of two further contributions by the writer, one on host range and the present one on host resistance. It is shown that some species of cultivated plants are totally resistant to invasion of their roots by the parasite such as *Arachis hypogaea, Crotularia retusa, C. spectabilis* and *Oryza sativa,* while in the case of others the degree of resistance is less complete, the larvae being able to enter the root tissues and even, in some plant species, such as, *Crotularia anagyroides, C. striata* and *C. usaramoensis,* develop to the adult stage and give rise to small numbers of ova despite the stunting influence of the host. A list of cultivated plants found in the course of these studies attacked by *Rotylenchulus reniformis* is also presented. It is considered that the root-knot nematode present in the Gold Coast belongs to a hitherto undescribed species in the genus *Meloidogyne.*

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Trichuris dipodomis, n. sp., from Ord's Kangaroo Rat

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Through the courtesy of Dr. Marietta Voge I have been able to study some whipworms collected from *Dipodomys ordii* Woodhouse by Dr. R. Holdenried. Hannum (1942) reported *Trichuris minuta* (Rud.) from *Dipodomys* in Arizona. However, *T. minuta* is usually a parasite of oppossums and doubts have been expressed elsewhere concerning the correctness of Hannum's identification (Read and Millemann, 1953). Study of the present material from the kangaroo rats shows that it is not *T. minuta* (Rud.) but a new species herein described. This casts further doubt on Hannum's identification.

Trichuris dipodomis n. sp.

Three males and three females available for study.

Male: 19.7 to 25.1 mm. long; esophageal region 8.7 to 14.1 mm. long; posterior thick portion of body 10.3 to 11.6 mm. long. Width at head about 0.043 mm.; at esophageal-intestinal junction .280 to .290 mm.; in posterior body 0.330 to 0.465 mm. Spicule 1.21 to 1.33 mm. long; 0.025 mm. wide at tip; 0.040 mm. wide in mid region; and 0.075 to 0.079 mm. wide at base. Spicule sheath extends 0.200 to 0.250 mm. from posterior end of body. Extended sheath campanuliform at tip, covered with coarse, scale-like spines distally which become smaller proximally. Junction of spicule sheath and cloaca 0.432 to 0.581 mm. from posterior end of body. Total cloacal length 1.70 to 1.74 mm. Ejaculatory duct 1.16 mm. long.

Female: 39.5 to 47.9 mm. long; esophageal region 15.9 to 25.7 mm. long; post-esophageal region 22.2 to 23.6 mm. long. Vulva at junction of esophagus and intestine. Anterior vulvar labium slightly projecting. Maximum width in hindbody 0.515 to 0.581 mm.; width in middle of esophageal region 0.166 mm. Ovejector 0.747 to 0.913 mm. long. Anus almost terminal. Eggs 0.058 to 0.065 by 0.028 to 0.029 mm.

Location: Intestine.

Host: *Dipodomys ordii* Woodhouse.

Locality: Airport, Santa Fe County, New Mexico.

Type specimens: U. S. National Museum Helminthological Collection No. 38035 (holotype male and allotype female).

Trichuris dipodomis most closely resembles *T. opaca* Barker and Noyes, as redescribed by Tiner (1950). The present species differs from *T. opaca* in having a much coarser spicule and in having a longer spicule sheath which is spinose in its extended distal portion. The females of *T. dipodomis* are about twice the size of those of *T. opaca*.

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Early Larval Stages of Two Cestodes from Elasmobranch Fishes*

Nathan W. Riser

Although a great number of infective stage larvae of phyllobothrioid and tetrarhynch cestodes have been reported and described, no complete life-histories are known for these groups of tapeworms, nor have any been experimentally demonstrated. Early larval stages of the tetrarhynch *Grillotia erinaceus* (van Ben., 1858) were described by Ruszkowski (1932, 1934) following experimental infections of planktonic copepods with actively swimming coracidia. Riser (1949, 1951) reported a similar study with the tetrarhynch *Lacistorhynchus tenuis* (van Ben., 1858); and in the 1949 abstract, also mentioned studies on the early stages of a species of *Acanthobothrium* later described as *A. hispidum* Riser 1955. Delay in the publication of the details of these studies resulted from an effort to complete the life-history of *L. tenuis* using material from *Mustelus canis* (Mitch.) on the Atlantic Coast, but the material used was the *Rhynchobothrium bulbiferum* of Linton, a form considered by Dollfus (1942) on the basis of morphology to be synonymous with *L. tenuis*. Although the eggs of this tapeworm had the same dimensions as those of *L. tenuis*, a distinct operculum was not visible, and coracidia were not obtained from any of the gravid proglottids secured during a summer's work. Even though these eggs never hatched, efforts were made to feed mature ones to microcrustaceans, but no infections were established.

During the course (1946-1950) of an investigation of the cestode parasites of elasmobranch fishes, eggs containing viable onchospheres were routinely fed to various microcrustaceans. Initially, planktonic copepods were used, but they could not be kept alive for more than two or three days in the laboratory and the splash-pool copepod *Tigriopus fulvus* (Fisher) was turned to, more or less as a last resort, and it was successfully infected by two species of worms. *T. fulvus* is very easy to rear under laboratory conditions, and withstands the rough handling necessary for microscopic examinations. However, the species cannot be a normal intermediate host, since, when infected copepods were fed to known proper second intermediate hosts, the exoskeleton of the crustacean did not tear nor break, and the procercoids were not liberated. Although this copepod was not collected where it could contact eggs or coracidia from elasmobranch cestodes, the splash-pools were in rocks which were usually covered with gulls, cormorants, pelicans, and other sea-birds which could possibly have transmitted cestode infections to them. Routine examination of each copepod to eliminate those already containing cestode larvae was adhered to, but no *T. fulvus* with any previous infection were encountered.

Superfamily Phyllobothrioidea Southwell, 1930

On October 7, 1948, hundreds of gravid proglottids of *Acanthobothrium hispidum* were found in the chyle in the spiral valve of a *Tetronarce californica* (Ayres). A number of these proglottids were picked out of the chyle and were rapidly washed in three changes of sea water and placed immediately into mineralized sea water. The proglottids ruptured within a few minutes, discharging streams of ripe eggs. It is noteworthy that the gravid proglottids of hyperapolytic tetraphyllideans do not rupture in the gut of the host, but instead, several minutes after they are in sea water. The eggs were 0.059-0.063 by 0.049-0.059 mm. in diameter and contained a round onchosphere 0.018 mm. in diameter.

*Contribution from the Biology Department of Fisk University, and the Hopkins Marine Station of Stanford University.

On the following day eighty-five parasite-free *Tigriopus fulvus* were placed in the dish containing the eggs. The *Tigriopus* were removed after 12 hours and placed in a beaker of sea water. Seventy-one of them were still alive at this time. Upon examining the copepods four days later, it was discovered that each contained from five to eighteen onchospheres encysted in the wall of the intestine, and in only nine were free larvae present in the haemocoele.

Fifteen days after they were infected, several of the copepods were teased apart. The procercoids which were free in the haemocoele were very active when released. They rapidly contracted and expanded. A huge apical organ was situated at the anterior end (Fig. 4), and a well developed cercomere was present at the posterior end. Two pairs of larval hooks were the most found in the cercomere. Calcareous bodies were very numerous and made it difficult to search for the hooks. These larvae measured about 0.15 mm. in length. Procercoids removed from cysts on the wall of the gut were shorter and fatter and were not very active. The excretory system was not visible in any of the procercoids which were examined.

At the time of the next examination, seven days later, all of the copepods were dead. I do not believe that any conclusions as to the nature of the procercoid scolex can be drawn from these observations since the period of observation was very short, and the results are yet to be confirmed.

The encystment of the majority of the procercoids in the wall of the intestine probably indicated that *Tigriopus* was an abnormal host, or that the onchospheres were not fully mature.

Order Trypanorhyncha Diesing, 1863

On January 13, 1948, a number of gravid proglottids of *Lacistorhynchus tenuis* were obtained from the spiral valve of *Triakis semifasciata* Girard. These proglottids were rapidly washed and then placed in a Stender dish filled with mineralized sea water. Shortly after being placed in the sea water, the proglottids ruptured and segmenting ova 0.047 mm. long by 0.026-0.028 mm. wide spiraled from them in streams. The effete proglottids were removed and the dish was placed in the dark. Four days later, coracidia began to be liberated from the eggs. The coracidia (Fig. 2) were 0.049 mm. in diameter and bore very long cilia over the entire surface. The onchospheres were pear-shaped, 0.037-0.039 mm. long with the maximum breadth of 0.029-0.033 mm. occurring in the region of the hooks. The embryonic hooks were 0.009-0.12 mm. in length. Several onchospheres were liberated from coracidia by the pressure of the coverslip as the water evaporated while they were being examined under the microscope. These onchospheres (Fig. 1) moved actively, drawing the part of the body bearing the hooks along as they wormed their way across the field of the microscope. No flame cells were visible, and very little cellular differentiation could be demonstrated.

One hundred *Tigriopus fulvus* were introduced to the dish of coracidia. Twenty-four hours later, the copepods were strained out of the infective culture and placed in a separate beaker of sea water. Almost all of the copepods were infected; many of them excessively as the culture of coracidia was very heavy. Nineteen larvae were counted in the haemocoele of one of the copepods.

The seven day-old procercoid was cigar-shaped, 0.225 mm. long and 0.045 mm. wide. No cellular or morphological differentiation was noticeable. At thirteen days, the cuticle was formed. Chalk bodies began to appear after the fourteenth day, and the excretory vessels appeared after the sixteenth day. By the eighteenth day, the anterior end was invaginated (Fig. 3).

This group of procercoids was studied for forty-one days after the copepod hosts were infected. There was little growth after the thirteenth day. Forty-one day old larvae averaged 0.3 mm. in length and 0.09 mm. in width. In heavy infections only one or two of the procercoids grew to this size, the others were about half this size. A cercomere was never observed in living material, the larval hooks lying in the vicinity of the excretory bladder. However, one 26 day old procercoid (Fig. 5) which was killed and fixed while still in the copepod, showed a peculiar posterior lobe bearing the hooks. This is reminiscent of the figure given by Ruszkowski (1934) of the oldest procercoid of *Grillotia erinaceus* which he observed. He also failed to observe a true cercomere.

Ten infected copepods were fed to a young *Clevelandia ios* (Jordan and Gilbert) on February 7, 1948. Ten more were fed to another young *Clevelandia* on the 13th, and five to another on the 22nd. The *Cevelandia* were examined on March 7, 1948. None were infected. This was expected since the faecal casts from the fish following each feeding had been examined and all twenty-five copepod skeletons still contained procercoids.

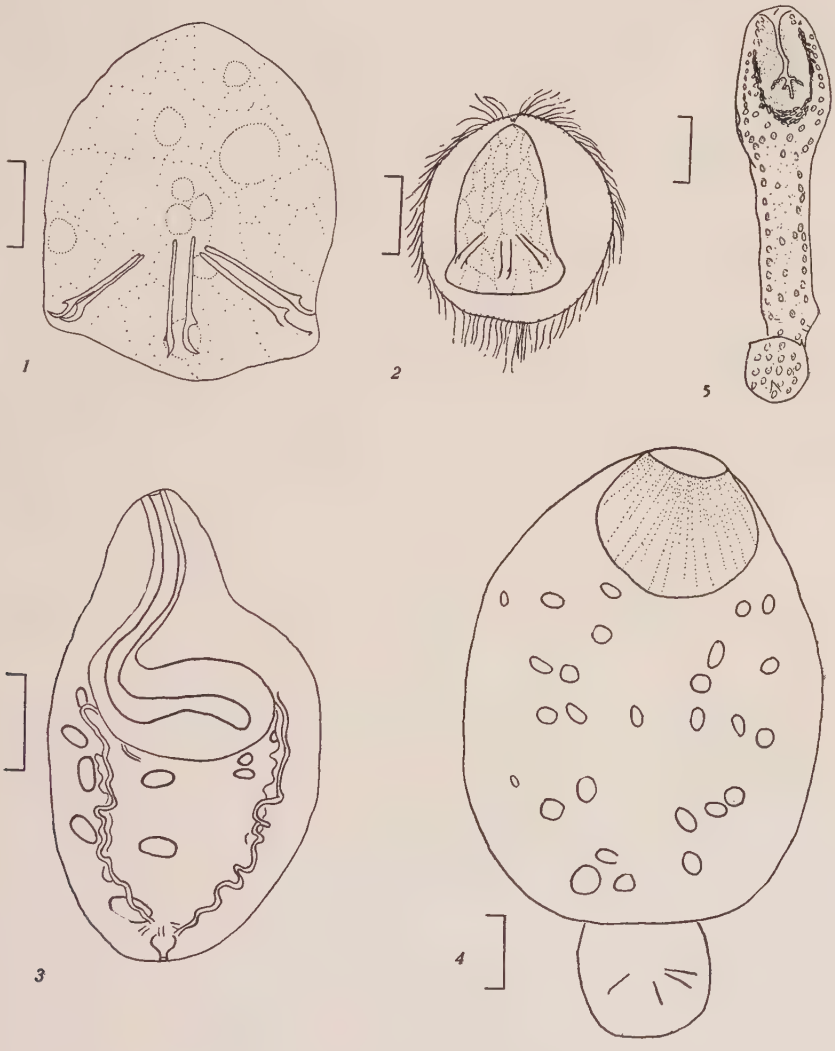
On June 16, 1948 another *Triakis* yielded many gravid proglottids of *L. tenuis*. These were handled in the same manner as the first lot. Coracidia appeared on June 21. Once again, *Tigriopus* was used for the experimental infections. Twenty-eight days after the infection, thirty infected copepods were fed to a young specimen of *Micrometrus minimus* (Gibbons). When this fish was sacrificed twenty-two days later, no worms could be found.

Another group of eggs were obtained on September 28, the coracidia began to appear on October 3rd. *Tigriopus* was again used for the intermediate host. One and one-half hours after being placed among the coracidia, free onchospheres could be seen in the haemocoeles of many of the copepods. A young *Oligocottus snyderi* Greeley was fed infected copepods twenty-eight days after the latter were infected. Upon examination three weeks later, no worms were found.

The eggs of *L. tenuis* are laid while segmenting, and four to five days are required for them to hatch. The history of the onchosphere and procercoid in the intermediate host is similar to that of other tetraphyllideans except that no scolex forms in the procercoid, but the region in which it is to develop invaginates. Dollfus (1942: 326, Fig. 241c) figures a young plerocercoid very similar to the oldest procercoids which were observed in this study, and thus, at least in this species, scolex formation takes place entirely in the plerocercoid stage.

In the detailed studies of tetrarhynch plerocercoids by Pintner (1893, 1896, 1930, 1931) much emphasis was placed on the histology of the excretory bladder, and the posterior end of the caudal appendage. There is no apparent difference between the bladder region of procercoids of *L. tenuis* and that region of the plerocercoids of that species and the other plerocercoids described by Pintner. Thus, there should be no cercomere in the procercoid stage, and the plerocercoids should bear the larval hooks in the vicinity of the excretory bladder. These hooks have not been observed, nor reported. Riser (1955) placed the tetrarhynchs in a superorder Trixenidea, one of the characters of which was the absence of larval hooks on the infective larvae. It is considered probable that an investigation of tetrarhynch plerocercoids with the purpose of discovering larval hooks in the region of the excretory bladder will demonstrate that these infective larvae bear hooks and that this character for

the superorder will be nullified. It is the author's regret that the tetrarhynchs which he collected were all given to Dr. R. Ph. Dollfus who is in the process of describing them, and no material remains available for examination at the present time.



All figures drawn with the aid of a camera lucida. The scale to the left of Figs. 3 and 5 = 0.05mm, of Figs. 2 and 4 = 0.02mm, of Fig. 1 = 0.01mm.

Fig. 1. Onchosphere of *Lacistorhynchus tenuis*.

Fig. 2. Coracidium of *L. tenuis*.

Fig. 3. Procercoid of *L. tenuis* from haemocoele of *Tigriopus fulvus* 21 days after infection.

Fig. 4. Procercoid of *Acanthobothrium hispidum* from haemocoele of *T. fulvus* 15 days after infection.

Fig. 5. Procercoid of *L. tenuis* from haemocoele of *T. fulvus* 26 days after infection.

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Paratylenchus dianthus, n. sp. (Nematoda, Criconematidae), a Parasite of Carnation*

W. R. JENKINS AND D. P. TAYLOR**

In February, 1955, root and soil samples were collected in three commercial greenhouses from carnations exhibiting poor growth. Routine examination for nematodes revealed that a new species of *Paratylenchus* was present in these samples.

Each of four pots containing three plants each of the carnation variety Sidney Littlefield were inoculated with approximately one thousand individuals of this species. After 50 days examination of the soil and roots from each pot revealed a population increase of 700 per cent. One thousand nematodes of *Paratylenchus* n. sp. inoculated into fallow pots failed to survive, indicating that the presence of a suitable host is necessary.

It seems probable that this species of nematode was one of the contributing factors to poor growth in the three greenhouse plantings sampled.

Paratylenchus dianthus, new species (Figure 1)

25 Females: 0.37 mm. (0.32-0.44 mm.); a = 21.7 (18.0-25.8); b = 4.2 (3.5-4.8); c = 12.7; V = 84% (80.5-87.1); stylet = 25.8 μ (20.6-28.9).

15 Males: 0.38 mm. (0.32-0.48 mm.); a = 24.2 (19.7-27.4); b = 4.0 (3.1-4.9); c = 14.2 (12.0-15.4); spicule = 24.7 μ (20.1-28.5).

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FEMALE.—Cuticle marked by transverse striae about 2.0 microns apart. Lateral field occupying about one-third of the body diameter and marked by four evenly-spaced incisures. The lip region is truncate, continuous with the body contour, and bears six lips. No amphids have been observed. A cervical papilla is located in the region of the nerve ring. The conspicuous excretory pore opens ventrally near the base of the esophageal isthmus. No phasmid has been observed on the female. There is an average of 44 post-vulval annulations

The buccal cavity is small and its base appears to serve as a guide for the slender, heavily nobbed spear. Dorsal esophageal gland opens into the lumen of the esophagus about 4.6 μ behind the base of the spear. There is a typically criconematoid middle bulb and valvular apparatus followed by a long and very narrow isthmus. There is no cardia between the somewhat pyriform basal bulb of the esophagus and the intestine. The intestine, the cells of which are filled with many small inclusions, ends in a short rectum and obscure anus.

The single outstretched ovary is quite long, usually reaching to the base of the esophagus. From a cap cell there arise several oögonia arranged in a double line. There is a spermatheca, containing a mass of sperm cells, at the anterior end of the uterus. Only one egg, which averages 38.7 μ by 10.9 μ, is observed in the uterus at a time. The vulva is a transverse slit flanked by lateral vulva membranes. No posterior uterine sac has been observed. Immediately behind the vulva, the body narrows markedly from 16-17 μ to 13 μ.

A ventral curving, as shown in the figure, is the normal relaxed position of the mature female.

MALE.—Males occur about one-sixth as frequently as females. A stomatostylet is entirely lacking in this sex. The poorly developed esophagus extends posteriorly from a very small buccal cavity. There is a much reduced middle bulb containing only a very small valvular apparatus.

A single outstretched gonad is attached to a pair of simple, ventrally curved, spicula. There is a thin gubernaculum which is curved ventrally except for a straight distal end. A sheath which surrounds the spicula protrudes from the anal opening. No hook-like process is present on the posterior margin of the sheath. The male tail is tapering and rounded and bears a phasmid midway between the anal opening and tail tip.

TYPE HOST.—Roots of cultivated carnation, *Dianthus caryophyllus*.

TYPE LOCALITY.—Rockville, Montgomery County, Maryland.

DIAGNOSIS.—With respect to males and their characteristics, 3 groups may be recognized in the genus *Paratylenchus*. Males are absent in the first group which includes *P. bukowinensis* Micoletsky, 1922, *P. nanus* Cobb, 1923, *P. anceps* Cobb, 1923, and *P. curvitata* van der Linde, 1938. In the two remaining groups males occur. In the second group, which includes *P. goodeyi* Oostenbrink, 1953, and *P. hamatus* Thorne and Allen, 1950, stylets are distinct. Males of the third group, containing *P. besoekianus* Bally and Reydon, 1931, *P. elachistus* Steiner, 1949, *P. minutus* Linford, 1949, and *P. macrophallus* (de Man 1880) Goodey, 1934, have indistinct or shadowy stylets or lack stylets altogether.

P. dianthus n. sp. males lack a stylet; therefore, this species is included in the third group. Certain morphological characteristics exist to differentiate males of *P. dianthus* from other species in this group. *P. besoekianus* has a truncated head, and like *P. elachistus* and *P. minutus,* is smaller than *P. dianthus*. *P. dianthus* males differ from those of *P. macrophallus* in that the

latter species has a truncated and offset head. Males of *P. dianthus* are of frequent occurrence as opposed to the rare incidence of males of *P. macrophallus*.

In many cases females of the genus *Paratylenchus* differ only slightly; therefore, a list of differences between *P. dianthus* n. sp. and the other species is given below:

P. bukowinensis and *P. besoekianus* have post-vulval uterine branches which are not present in *P. dianthus*.

The egg of *P. nanus* is much larger (60 x 20 μ) than that of *P. dianthus* (38.7 x 10.9 μ). *P. nanus* differs further in lacking lateral membranes at the vulva, in the absence of a marked reduction in body diameter posterior to the vulva, and in the non-bulging wall of the esophagus around convolutions of the esophageal lumen.

The striations of *P. anceps* are only 1 μ apart; there are but 2 lines in its lateral field; and it is a smaller species than *P. dianthus*.

Three other species, *P. besoekianus, P. elachistus,* and *P. minutus,* are also smaller than *P. dianthus*.

P. elachistus does not have a truncated head and its longer ovary extends anteriorly as far as the middle bulb.

A lateral vulval membrane is lacking in *P. minutus* and it has a smaller stylet.

The head of *P. curvitata* is distinctly offset while that of *P. dianthus* is continuous. *P. hamatus* has a cardia, a non-truncate lip region, and there is no change in body diameter posterior to the vulva. In addition 2 eggs were illustrated in the uterus of *P. hamatus*.

The stylet of *P. goodeyi* is about twice as long as that of *P. dianthus* and the former species is more stout.

Dimensions of females of *P. dianthus* fall within the limits of those reported for *P. macrophallus,* but the new species is far less variable. Furthermore, in *P. macrophallus,* lateral vulval membranes are lacking; there is no post-vulval reduction in body diameter; and there is a slight constriction setting off the lip region.

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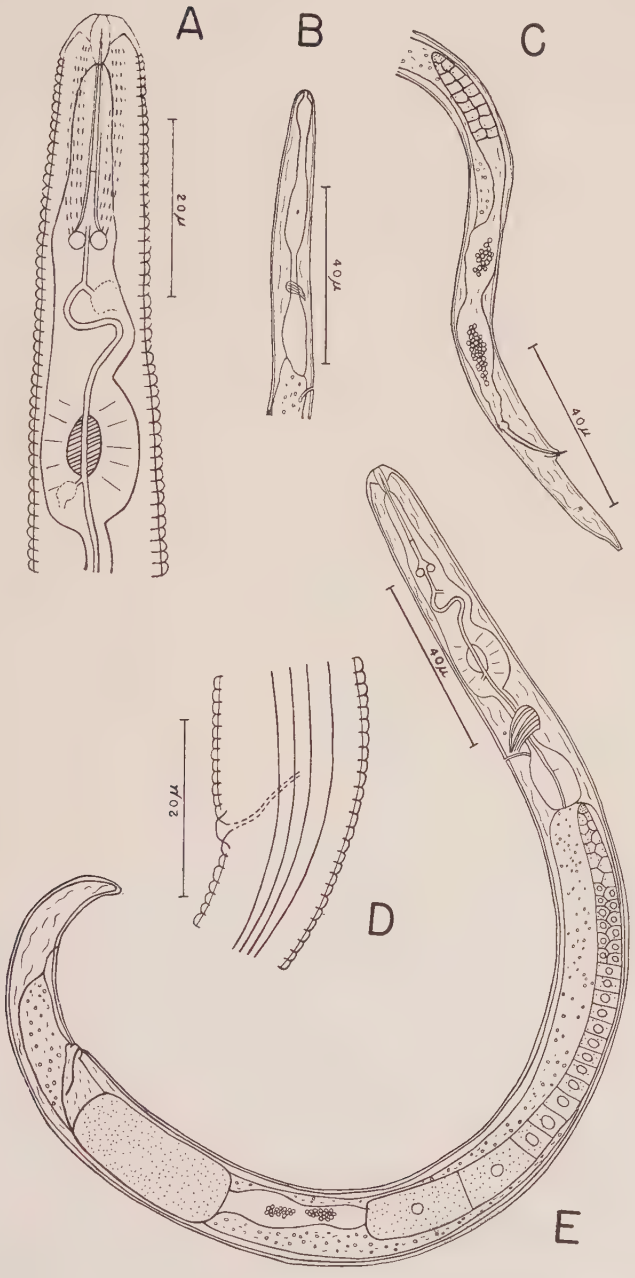

Fig. 1. *Paratylenchus dianthus*, n. sp. A—Anterior portion of female; B—Anterior portion of male; C—Posterior portion of male; D—Vulvar region showing lateral field; E—Mature female showing relaxed position.

Nacobbus batatiformis, n. sp. (Nematoda: Tylenchidae), Producing Galls on the Roots of Sugar Beets and Other Plants*

Gerald Thorne and M. L. Schuster**

Sugar beet roots collected by the junior author in 1949 from a field near Mitchell, Nebraska, bore galls similar to those produced by species of *Meloidigyne,* but the nematodes within them obviously belonged to a different group. The collection was forwarded to A. L. Taylor who assigned the pathogen to the genus *Nacobbus.* Some of the specimens were then submitted to the senior author who determined that the species differed from the two previously described; *N. abberans* (Thorne, 1935) and *N. dorsalis* Thorne and Allen, 1944.

Surveys of the North Platte River Valley, conducted in 1953 and 1954, revealed that *Nacobbus batatiformis,* is present in Scotts Bluff, Sioux and Morrill Counties of Nebraska. Included in these surveys were 125 sugar beet fields, of which 32 percent were infested. Usually the infested fields were sandy, coarse-textured soils. Specimens were also found on sugar beets from Windsor, Colorado, collected by Russell Nelson, and C. W. McBeth reported them from sugar beets near Torrington, Wyoming.

Host Range of *Nacobbus batatiformis*

Host range studies ware conducted by examining plants collected from infested fields and experimental plots, supplemented by greenhouse tests of plants selected to include the major crop plants grown in the locality as well as members of various families. The presence of females producing viable eggs was the criterion by which plant species were determined to be susceptible.

The degree of root distortion and galling varied greatly among the infected plants and preliminary observations indicate that certain hosts are more favorable than are others to the development of *N. batatiformis.* Females from the succulent tissues of sugar beets are usually plump and well developed while those from the roots of *Salsola, Chenopodium* and similar plants are deformed and misshapen by the pressure of the hard, woody tissues. Differences in nutrition may also be responsible for variations observed in the form and size of individuals from the various hosts.

A total of 74 plant species, including 92 varieties, representing 15 families in the Angiosperms, was tested for their reaction to *N. batatiformis.* All species tested of the Chenopodiaceae, Cruciferae, Cactaceae, and Zygophyllaceae gave susceptible reactions, whereas those of the Gramineae, Liliaceae, Malvaceae, Iridaceae, Amaranthaceae, and Convolvulaceae appeared to be non-susceptible. Species of the Leguminosae proved to be non-susceptible with the exception of *Pisum sativum* on which rather small galls were produced. Some species of the Cucurbitaceae, Umbelliferae, Compositae and Solanaceae were resistant while others were susceptible.

Susceptible Species

Cactaceae: Barrel cactus, *Mamillaria vivipara* (Nutt.) Haw.; brittle cactus, *Opuntia fragilis* (Nutt.) Haw.; prickly pear, *O. tortispina* Nutt.

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CHENOPODIACEAE: *Beta vulgaris* L. (Early Blood Turnip); Sugar beet, *B. vulgaris* L. (G.W. 304R, Klein Wanzleben); Swiss chard, *B. vulgaris cicla* L. (Fordhook Giant); Mangel, *B. vulgaris macrorhiza* L. (Mammoth Long Red); Lamb's-quarters, *Chenopodium album* L.; Kochia, *Kochia scoparia* (L.) Schrad.; Russian thistle, *Solsola kali* var. *tenuifolia* Tausch; Spinach, *Spinacia oleracea* L. (Giant Nobel).

CRUCIFERAE: Rutabaga, *Brassica napbrassica* Mill. (American Purple Top); Mustard, *B. nigra* (L.) Koch. (Chinese Broad Leaf); Kale, *B. oleracea viridis* L. (Dwarf Green Scotch); Broccoli, *B. oleracea botrytis* L. (Early Snowball); Cabbage, *B. oleracea gongylodes* L. (Early Purple Vienna); Brussels sprouts, *B. oleracea gemmifera* L. (Long Island Improved; Chinese cabbage, *B. pekinensis* (Lour.) Rupr. (Pe-Tsai); Turnip, *B. rapa* L. (Purple White Top Globe); Stock, *Matthiola* sp.; Radish, *Raphanus sativus* L. (Early Scarlet Turnip).

ZYGOPHYLLACEAE: Puncture vine, *Tribulus terrestis* L.

COMPOSITAE: Gaillardia, *Gaillardia pulchella* L. (Indian Chief); Lettuce, *Lactuca sativa* L. (Black Seeded Simpson); Salsify, *Tragopogon porrifolius* L. (Mammoth Sandwich Island).

CUCURBITACEAE: Pumkin, *Cucurbita pepo* L. (Early Sugar); Cucumber, *Cucumis sativus* L. (Improved Long Green).

SOLANACEAE: Tomato, *Lycopersicon esculentum* Mill. (Earliana, Marglobe, Sioux, Bonny Best, John Baer); Tomato, *L. peruvianum* (L.) Mill. (Acct. No. 903); Eggplant, *Solanum melongena* L. (Black Beauty).

LEGUMINOSAE: Pea, *Pisum sativum* L. (Alaska, Little Marvel).

UMBELLIFERAE: Carrot, *Daucus carota* L. (Danvers Half Long, Imperida).

NON-SUSCEPTIBLE SPECIES

COMPOSITAE: Safflower, *Carthamus tinctorius* L. (N1, N3, N8, N852, N10); Endive, *Cichorium endivia* L. (Florida Deep Heart); Cosmos, *Cosmos* sp.; Sunflower, *Helianthus annuus* L.; Marigold, *Tagetes erecta* L. (Sunsets Giants Mixed); Zinnia, *Zinnia elegans* Jacq. (Red Riding Hood, California Giant).

CUCURBITACEAE: Squash, *Cucurbita maxima* Duch. (Table Queen); Cantaloupe, *Cucumis melo* L. (Hales Best and Rocky Ford); Watermelon, *Citrullus vulgaris* Schrad. (Dixie Queen, Blacklee Wilt Resistant).

SOLANACEAE: Pepper, *Capsicum* sp.; Potato, *Solanum tuberosum* L. (Progress, Bliss Triumph, Red Warba, Katahdin, White Rose, Yampa).

LEGUMINOSAE: Peanut, *Arachis hypogaea* L. (Spanish 146, Small Spanish); Soybean, *Glycine max* (L.) Merr. (Bansei); Sweet peas, *Lathyrus odoratus* L. (Cuthbertson's Heat Resistant); Alfalfa, *Medicago sativa* L. (Ranger): Sweetclover, *Melilotus officinalis* (L.) Lam. (Griswold's No. 1854); Tepary bean, *Phaseolus acutifolius latifolius* Freem. (No. 5); Bush lima bean, *Phaseolus limensis* Macf. (Allgreen); Bean, *Phaseolus vulgaris* L. (U.I. No. 123, Red Kidney, Kentucky Wonder).

UMBELLIFERAE: Dill, *Anethum graveolens* L. (Long Island Mammoth).

AMARANTHACEAE: Pigweed, *Amaranthus retroflexus* L.

CONVOLVULACEAE: Morning-glory, *Ipomoea tricolor* Cav. (Heavenly Blue, Pearly Gates).

GRAMINEAE: Oats, *Avena sativa* L. (Arlington); Brome, *Bromus inermis* Leys. (Lincoln); Barley, *Hordeum vulgare* L. (Velvon 11); Rye, *Secale cereale* L. (Abruzzi); Sorghum, *Sorghum vulgare* L. (Leoti Red); Wheat,

Fig. 1. Sugar beet infected with *Nacobbus batatiformis*.

Triticum vulgare L. (Coastal); Popcorn, *Zea mays* L. (White Japanese Hulless); Sweet corn, *Zea mays* L. (Golden Cross Bantam).

Iridaceae: Gladiolus, *Gladiolus* sp.

Liliaceae: Onion, *Allium cepa* L. (Yellow Sweet Spanish, White Sweet Spanish); Asparagus, *Asparagus officinalis* L. (Washington Rust Resistant).

Malvaceae: Okra, *Hibiscus esculentus* L. (Clemson spineless); Cotton, *Gossypium hirsutum* L. (Bowden 416).

Host relationships and pathological histology will be discussed in a paper published by Schuster and Thorne in the Proceedings of the American Society of Sugar Beet Technologists (1956).

Life Cycle of *Nacobbus batatiformis* in Sugar Beets

Since the sugar beet is the most economically important known host of *Nacobbus batatiformis*, studies on the life cycle have largely been made with this crop plant. But the wide host range of taxonomically diverse plant species indicates that considerable variation in the life cycle may be expected when it is studied in detail on the various hosts. Doubtless there will be only one generation on short-lived annuals while on perennials there may be two or more. And under the semi-desert conditions of western Nebraska, activity on native hosts must necessarily be restricted to relatively short periods when moisture is present, while on irrigated hosts the life cycle may be completed three or four times since favorable moisture conditions exist throughout the season.

Soil samples collected from a sugar beet field in mid-March contained a few preadults only; no larvae, males or young females were present. However, the complete destruction of young sugar beets over wide areas just after thinning indicated that far greater numbers of nematodes were involved than the few preadults found in the soil samples, and it is assumed that eggs overwinter as do those of *Meloidogyne* under similar conditions.

Larvae of *N. batatiformis* emerging from eggs have blunt, rounded tails and closely resemble young *Pratylenchus* spp. Although several hundred larvae were extracted from the eggs by crushing, none was found to possess a pointed tail as recorded by Thorne and Allen for the first stage larvae of *N. dorsalis*. Probably it was a larva of *N. batatiformis* which Cobb, 1918 described and figured as a blunt-tailed larva of the sugar beet nematode, *Heterodera schachtii* Schmidt. Cobb's specimen came from

"Colorado" and the validity of this assumption is substantiated by the fact that sugar beet roots collected near Windsor, Colorado, in 1954 contained *N. batatiformis* in association with *H. schachtii.*

When the larvae enter small sugar beet roots they establish themselves in favorable locations and often a dozen or more will be grouped in one small area. Frequently three or four will be found slightly behind the root tip. When feeding begins, the cells about the central cylinder increase greatly in size and as the gall develops, the affected portions become granular and later yellow colored. Two molts occur during this feeding period and at one stage the immature nematodes often assume an "open C" form, averaging 0.5 mm in length, after which they grow to 0.65-0.8 mm in length, when they usually are found coiled in the galls (Fig. 2D, E). At this time the small roots generally die and the preadult nematodes migrate into the soil where at least part of them make their final moult and become males or juvenile, active females. Copulation might be expected to occur at this time, but among those examined not one of the young females from soil was spermatized.

After leaving the small roots in which they develop the immature females move to other roots, usually larger ones, and again establish themselves with their heads buried in the cells surrounding the central cylinder. Hundreds of these cells become enlarged and frequently 3 to 6 females will be found grouped in a single gall. As the gall develops, the posterior portion of the female extends toward the periphery and an opening is formed in the gall through which the eggs are discharged into a gelatinous matrix extruded by the nematode (Fig. 3 D). Occasionally males are found entangled in this gelatinous mas, indicating that the female is fertilized after gall formation has begun. The chamber in which the female develops is lined with a brittle coating which remains after the female dies.

Large galls frequently contain no living females; only the masses of tough darkened tissues mark the spots in which they developed. Galls usually bear great numbers of small rootlets, sometimes 20 to 50 occurring on one. In advanced stages, sections of roots between galls and even portions of the beet itself will bear many of these small rootlets (Fig. 1).

Nacobbus batatiformis, n. sp.

Female: Length 0.7-1.4 mm. Body varying greatly in form, typically spindle-shaped but often with irregular expansions and constrictions produced by pressure of root tissues during development. Sometimes the head and neck are depressed into the anterior end of the body until almost indistinguishable. Posteriorly the body generally is somewhat conoid to the truncated terminus near which the vulva and anus are located. (Fig. 2 F, G, H, and Fig. 3 B, C). Spear 15-18μ long with small basal knobs. Lip region usually elevated slightly above the head contour. Corpus of esophagus strongly developed, frequently irregular in form and set off from the median bulb by a narrow constriction. Median esophageal bulb strongly developed with conspicuous radial musculature. Esophageal glands forming a broad, thick lobe, usually pressed against the anterior end of the intestine which is packed with coarse, refractive granules. Nuclei of intestinal cells frequently conspicuous. From the deeply depressed, slit-like vulva the muscular vagina extends forward to the uterus which ends in an ovate or pyriform spermatheca, containing hundreds of spermatozoa. Immediately behind the cap cell the oocytes within the ovary number about 12 to a

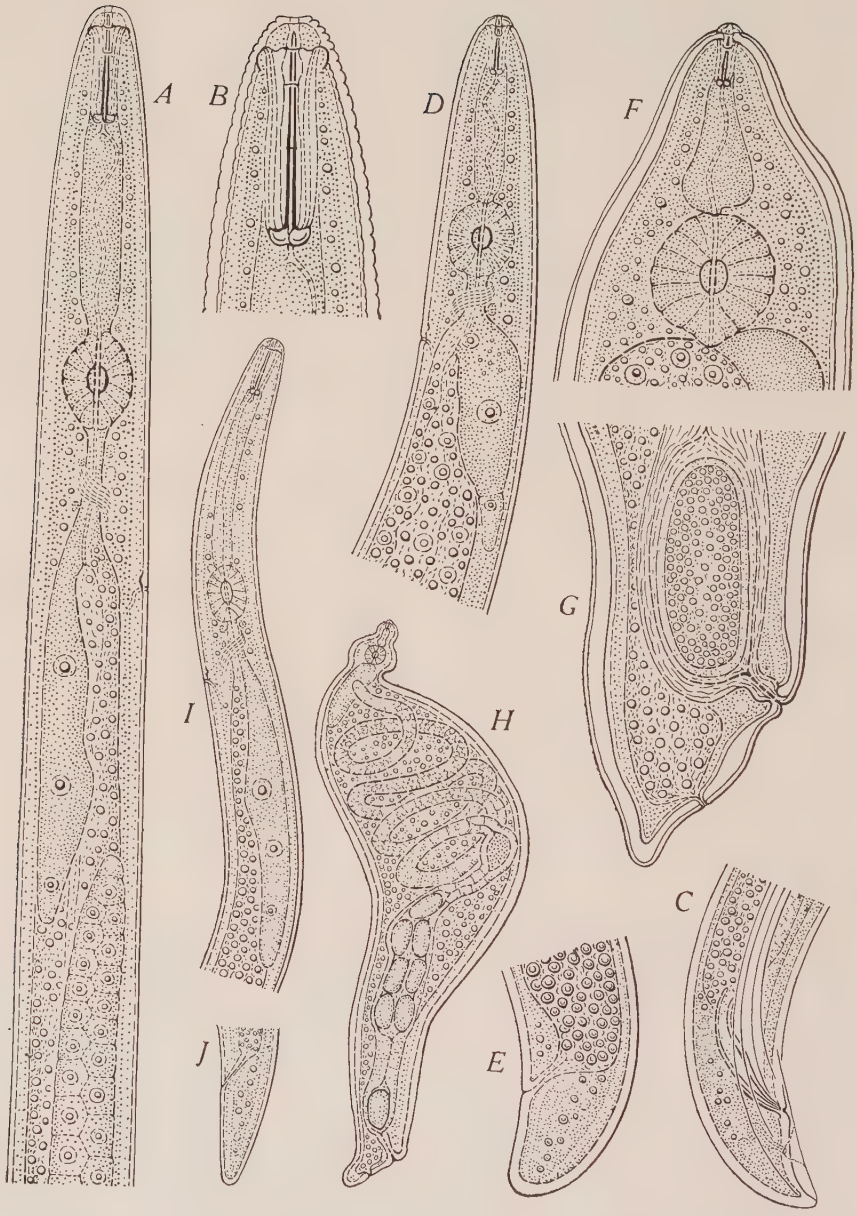

Fig. 2. *Nacobbus batatiformis*. A, B, C, Anterior portion, head and tail of male; D, E, Anterior and posterior portions of preadult female; F, G, Anterior and posterior portions of adult female; H, female; I, J, Anterior and posterior portions of larva.

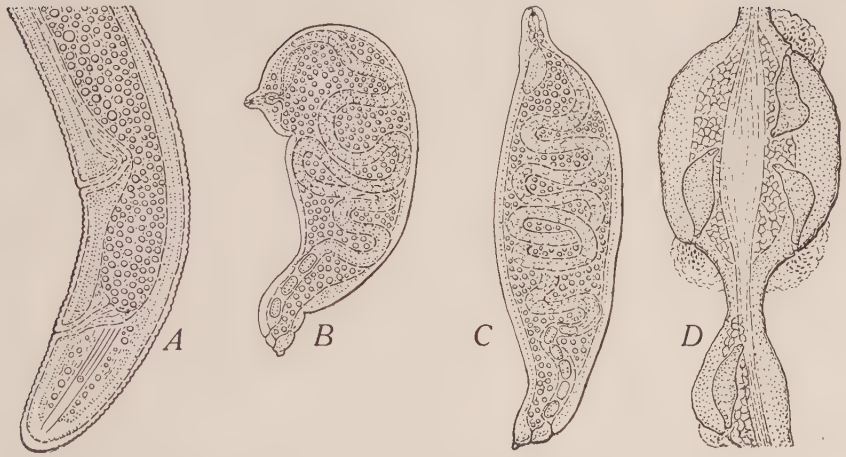

Fig. 3. *Nacobbus batatiformis:* A, posterior portion of young female; B, C, Variations in form of female; D, Sketch of gall on sugar beet root showing location of females, and gelatinous masses into which eggs are deposited.

circumference, arranged about a slender rachis, and as they develop the number is reduced to about eight, then six and finally four when they become fully developed. The thin-walled eggs average 49 x 83μ, and are deposited before segmentation. Usually the ovary has several more convolutions than illustrated (Fig. 2 H).

MALE: 0.8-1.2 mm; a = 32; b = ?; c = 35-45; T = 62-78%.

Lip region continous with body contour, bearing four annules in addition to the labial disc. Lateral fields marked by four incisures appearing as bright refractive lines. Labial framework hexaradiate, heavily sclerotized. Spear 20-25μ long with strong basal knobs which are slightly cupped anteriorly. Median bulb of esophagus about half as wide as neck. Esophageal glands forming a lobe extending back a distance equal to three to five times the body width. Hemizonid adjacent to excretory pore. Deirids not observed. Testis usually extending forward to a point three or four body-widths posterior to the esophageal gland lobe, but occasionally it reaches midway of the lobe. Spicula averaging 26μ long; gubernaculum a simple trough, about one-fourth as long as the spicula. Bursa enveloping the short arcuate tail. Phasmid opening near base of bursa, slightly posterior to middle of tail. (Fig. 2 A, B, C).

LARVA: 0.32-0.38 mm; a = 18; b = 4.5; c = 15.

Cuticle marked by very fine transverse striae. Lateral field outlined by two fine, bright lines. Lip region, spear and general body form resembling those of a young *Pratylenchus*. Tail bluntly rounded, even on larvae removed from eggs prior to hatching (Fig. 2 J). First stage larvae with pointed tails were not observed as in *Nacobbus dorsalis*. Lobes containing esophageal glands about as long as body width at the time of hatching, but they develop rapidly and soon appear as illustrated (Fig. 1 I). Genital primordium a single cell with a well-developed nucleus when the larva emerges from the egg.

Diagnosis: *Nacobbus* with the above measurements and general description. Median bulb and corpus of female esophagus strongly developed, posterior portion of body not elongated. Vulva-annus distance of infective female slightly longer than tail length (Fig. 3 A); phasmid near middle of tail. Males generally assume an elongated U-shape when relaxed by gradual heat.

Key to Species of *Nacobbus*

1. Adult female anteriorly spheroid or ovate, then slender, elongated; infective female with vulva-anus distance equal to about three times the tail length; male tail about one and one-half times as long as anal body diameter ..... *dorsalis* Thorne and Allen.
   Adult female spindle-shaped or irregular in form; infective female with vulva-anus distance one to two times the tail length; male tails about as long as anal body diameter 2
2. Adult female with strongly developed median bulb and corpus; infective female with vulva-anus distance about equal to tail length and with phasmids near middle of tail ..... *batatiformis*, n. sp.
   Adult female without strongly developed median bulb and corpus; infective female with vulva-anus distance twice tail length, and phasmids near terminus ..... *aberrans* (Thorne).

These three species of the genus *Nacobbus* represent very closely related forms which have evolved from common ancestry in three arid geographical areas which have long been isolated by high mountain ranges: *N. batatiformis* in the Great Plains area east of the Rocky Mountains, *N. aberrans* in the Great Basin between the Rockies and the Sierra Nevada, and *N. dorsalis* west of the Sierra Nevada. Adaptation to host plants has accompanied this isolation with *N. dorsalis* transferring from some unknown native host to alfileria, *Erodium cicutarium* (L.) L'Her, an introduction from Europe. *N. aberrans* became adapted to a common native shrub of the Great Basin, *Atriplex confertifolia* (Torr. & Frem.) S. Watts. And *N. batatiformis* became adapted to a wide variety of host plants, with three species of cactus as apparently the principal native reservoirs from which the nematodes transferred to sugar beets and other crop plants when the land was placed under cultivation. Similar omniverous habits have not been observed for *N. dorsalis* and *N. aberrans,* both of which inhabited large areas of land which now are producing the same crops on which *N. batatiformis* thrives.

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Two new species of microphallid trematodes of the genus *Levinseniella* from charadriiform birds

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A single specimen of a Red-backed Sandpiper was collected on October 7, 1950 in Ottawa Co., Ohio. Upon examination it was found that the bird harbored four trematodes which could be assigned to the genus *Levinseniella* Stiles and Hassall, 1901. Uuder similar circumstances four distomes, belonging to the same genus, were found in a Sanderling collected near Salina Cruz, Oaxaca, Mexico on August 30, 1955.

Distomes in the genus *Levinseniella* are typical microphallids, but they can be differentiated easily from the other genera in the family by the presence of four muscular pockets which are associated with the terminal male genitalia. In this family it is especially welcome to have a genus which appears to be a natural group based on a unique and prominent morphological structure. This is in contrast to the several genera which are of uncertain status. Rankin (1939) reviewed the genus and briefly outlined the characters of the species he recognized. Since that time Yamaguti (1939), Young (1949), and Etges (1953) have added to the genus.

The usual techniques were used to prepare the slides for study. All measurements are in millimeters from specimens mounted in damar. This study was carried out while the author was a Muellhaupt Scholar at the Ohio State University and it was supported by a Grant-in-Aid from the Sigma Xi-Resa Research Fund. The author is indebted to Emmett W. Price and Allen McIntosh for their Cooperation in making specimens available for study at the Agricultural Research Center, Beltsville, Maryland.

Levinseniella leptophallus, n. sp. (figure 1)

Diagnosis: with the characters of the genus. Relatively small, linguiform distomes with completely spinose cuticula, heaviest being anterior. Body 0.76-0.83 long and 0.32-0.34 wide at broadest point. Oral sucker 0.083-0.099 wide and 0.080-0.093 long. Prepharynx 0.02 long. Pharynx 0.057-0.067 wide and 0.038-0.045 long. Esophagus 0.11-0.20. Ceca short, with heavy irregular epithelium containing large nuclei. Elongate testes, about 0.06 long located along lateral margins. No cirrus sac. Seminal vesicle 0.089-0.11 long by 0.039-0.045, lying anterior to acetabulum almost on midline. Pars prostatica well developed 0.022-0.026 by 0.096, surrounded by well developed prostate cells. Ductus ejaculatorius muscular, narrow, entering male pocket through narrow papilla, 0.005-0.011 in diameter. Papilla enters male pocket mediad to muscular pockets with thin walls, 0.002-0.0028, lacking ornamentation. Female pocket separate, highly diverticulated, lacking muscular wall. Ovary elongate lying lateral and dorsal to acetabulum. Number of vitelline follicles obscured by eggs, situated laterally and in posterior sixth. Eggs numerous 0.019 by 0.009.

Host: Sanderling *(Crocethia alba).*

Locality: Near Salina Cruz, Oaxaca, Mexico.

Type Specimen: Holotype in the Helminthological Collection of the U.S. National Museum, No. 38137.

Levinseniella carcinidis Rankin, 1939, *L. charadriformis* Young, 1949, and *L. amnicolae* Etges, 1953 have been described with pockets which are

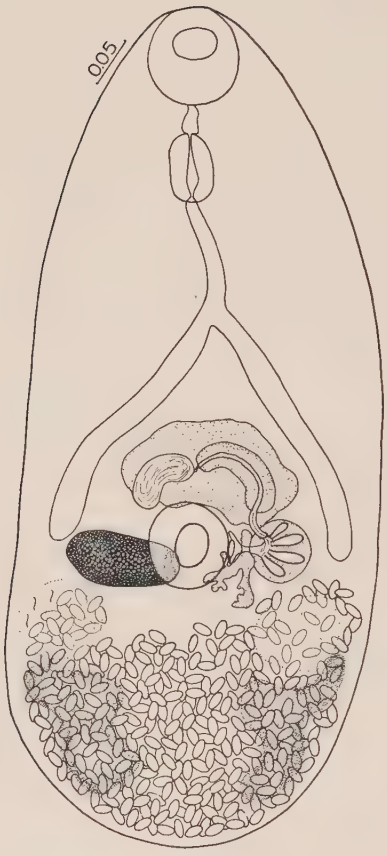

Figure 1. Ventral aspect of *Levinseniella leptophallus* drawn with the aid of a microprojector, but details added freehand.

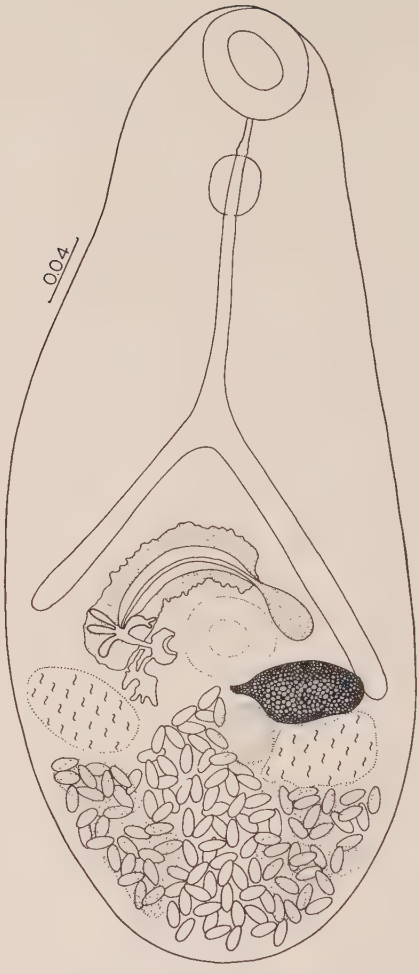

Figure 2. Dorsal aspect of *Levinseniella gymnopocha* drawn with the aid of a microprojector, but details added freehand.

not provided with scleratized structures, hooks, or ribs. *Levinseniella leptophallus* is similar to these species in some respects, can be differentiated from them by the nature of the female pocket. The female pocket is highly diverticulated, nonmuscular and it is separated from the male pocket by being directly connected to the genital atrium.

Levinseniella gymnopocha n. sp. (figure 2)

Diagnosis: with the characters of the genus. Small, claviform distomes with the spines arranged quincunx fashion, being heavier anteriorly, extending to region of acetabulum. Body 0.53-0.75 long and 0.22-0.29 wide at

level of acetabulum. Oral sucker 0.057-0.080 wide and 0.068-0.070 long. Prepharynx 0.009-0.025 long. Esophagus 0.09-0.1 long. Ceca short, with heavy, irregular epithelium containing large nuclei. Testes subcircular, about 0.05-0.07 in diameter located along lateral margins. No cirrus sac present. Seminal vesicle 0.05-0.07 lying lateral and slightly anterior to acetabulum. Pars prostatica well developed, 0.017 by 0.045, surrounded by prostate cells. Male papilla small, very inconspicuous opening into male pocket between muscular pockets with thin-walls, 0.0013 and lack ornamentation. Female pocket separate, highly diverticulated and without muscular walls. "Seminal receptacle" large, 0.02, in diameter, located mediad to right testis. Ovary dextral, subellipsoidal, lying mediad to end of cecum. Vitelline follicles, about eight, arranged laterally at posterior. Eggs numerous, 0.018-0.022 by 0.009-0.012.

Host: Red-backed Sandpiper *(Erolia alpina pacifica)*.

Locality: Ottawa County, Ohio, U.S.A.

Type Specimen: Holotype in the Helminthological Collection of the National Museum, No. 38138.

Levinseniella gymnopocha is most closely related to *L. leptophallus,* described above, but can be considered unique by the location of the entrance of the male papilla into the male pocket. In *L. gymnopocha* the papilla enters the male pocket between two of the muscular pockets while with the other species it enters mediad to the four pockets.

As with many of the other microphallids, it appears that the nature of the terminal male genitalia is useful as a taxonomic character. These worms are quite small and frequently it is difficult to make out the details, but it is essential that one determine these features if we are to avoid chaos in this genus.

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NEMATOLOGICA

International Journal of Nematological Research

A quarterly, totalling approximately 320 pages per volume (calendar year), devoted to nematology "except . . . . . medical and veterinary subjects." Both the above quotation and the names of the Advisory Editors suggest that it will deal primarily with free-living and plant parasitic nematodes. A. L. Taylor and M. W. Allen of this Society are the American representatives of the Board of Advisory Editors. The Editor-in-Chief is Dr. J. H. Schuurmans Stekhoven; the Secretary of the Editorial Board is Dr. P. A. van der Laan (Marterlaan 18, Bennekom, The Netherlands; and the Publisher is E. J. Brill (Leiden, The Netherlands). Volume 1, Number 1 appeared January 1956. Price: Gld 28.00 per volume (approximately $7.50).

Carneophallus muellhaupti, n. sp., a Microphallid Trematode from the Sanderling from Southern Mexico

William H. Coil
The Ohio State University

During the summers of 1954 and 1955, a number of Sanderlings were collected near Salina Cruz, Oaxaca, Mexico, and examined for their helminth parasites. Cyclophyllidean tapeworms were commonly found, and in 1955 some microphallid trematodes were obtained. The host *(Crocethia alba)* is a cosmopolitan shore bird nesting over much of the Northern Hemisphere and wintering along coast lines as far south as Capetown. It harbors a large variety of helminths including some other microphallids.

The family Microphallidae has received considerable attention during the past two decades, and in spite of many comprehensive studies, there is much to be gained in our understanding of this family. The genus *Carneophallus* Cable and Kuns, 1951 was erected mainly on the basis of the possession of a large, lobed genital papilla; and the distome described here can be assigned to this genus on the basis of this character

The specimens were fixed in corrosive acetic, stained with Harris' haemotoxylin, cleared in terpineol, and mounted in damar. This study was carried out while the author was a Muellhaupt Scholar in The Ohio State University and was aided by a Grant-in-Aid from the Sigma Xi RESA Research Fund. The author is indebted to Emmett W. Price and Allen McIntosh for their cooperation in making specimens available for study at the Agricultural Research Center, Beltsville, Maryland.

This specimen is named in honor of Mary S. Muellhaupt whose generous grant has made my study of mexican helminths possible.

Specific Diagnosis (figure 1, all measurements in millimeters): with the characters of the genus. Small distomes, pyriform to linguiform in shape with a tendency to become concave ventrally in fixed specimens. Body 0.32-0.37 long and 0.28-0.31 wide at level of the uterus. Oral sucker 0.049-0.061 wide, with papillae; acetabulum 0.049-0.059 wide. Prepharynx very short, 0.004. Pharynx 0.015-0.028 wide and 0.019-0.031 long. Esophagus moderately long. Ceca short, with heavy, irregular epithelium containing large nuclei. Internal organs plastic, very variable in shape, size and disposition. Testes circular, smooth about 0.065 in diameter. Seminal vesicle huge, elongate filled with sperm, occupying position mediad to ovary and right cecum. Prostate cells numerous, of irregular shape, filled with hyaline or finely granular cytoplasm. Genital pore sinistral, variable in size, with thin band of muscle fiber probably functioning as sphincter. Muscle fibers radiating from genital pore. Genital atrium large, thin-walled, almost filled with male "papilla" which is large, up to 0.091 long, and irregularly lobed. Ductus ejaculatorius courses through main lobe and opens to exterior some distance subterminally. No ornamentation observed on male "papilla." Elongate ovary dextral, just posterior to cecum. Vitelline follicles not obvious because of eggs; occupy area posterior to testes. Convoluted uterus occupies area posterior to acetabulum. Eggs very numerous 0.015 by 0.009.

Host: Sanderling *(Crocethia alba,* Scolopacidae*)*.

Locality: In the region of Salina Cruz, Oaxaca, Mexico.

Type Specimen: Holotype in the Helminthological Collection of the U.S. National Museum, No. 38155.

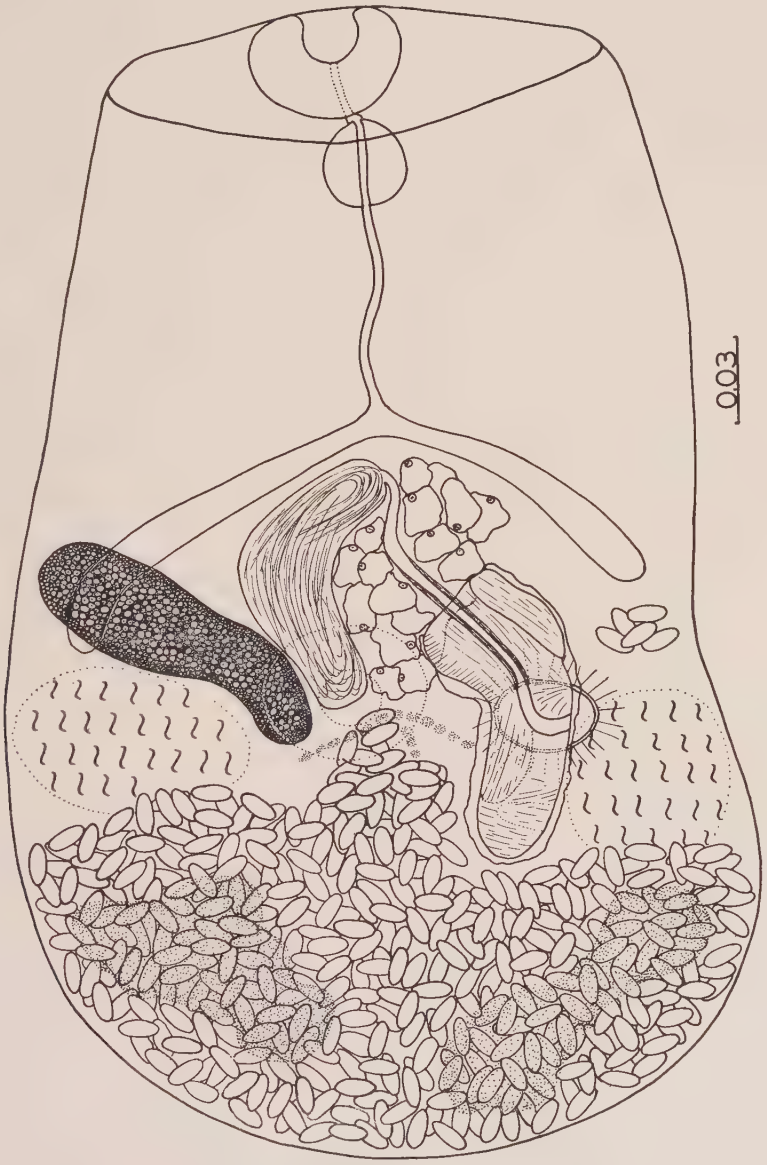

Figure 1. Ventral aspect of holotype. Drawn with the aid of microprojection; details added freehand. The anterior end is bent so that the oral sucker is seen *en face*.

Carneophallus muellhaupti can be differentiated from the other species in the genus (*C. trilobatus* Cable and Kuns, 1951 and *C. pseudogonotylus* (Chen, 1944) Cable and Kuns, 1951) by the relatively slight lobation of the male papilla and also by the placement of the opening of the ductus ejaculatorius. The distome described here appears to be an intergradation between *Spelotrema papillorobusta* Rankin, 1939 and *C. trilobatus*.

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Comparative Morphological Studies on the Soybean Cyst Nematode, *Heterodera glycines* and the Clover Cyst Nematode, *H. trifolii* (Nematoda: Heteroderidae)*

HEDWIG HIRSCHMANN

In January, 1955, Winstead, Skotland and Sasser reported the occurrence of the soybean cyst nematode, *Heterodera glycines* Ichinohe, 1952, in North Carolina. The nematode has been recorded previously only from Japan (Hokkaido, Tohoku, Hokuriku and Kanto) (Ichinohe, 1952, 1953, 1955), Korea (Yokoo, 1936) and China (Manchuria) (Nakata and Asuyama, 1938) as the cause of "yellow dwarf," a disease of soybean, *Glycine max* (L.) Merrill. *H. trifolii* Goffart, 1932, has been reported from several locations in the United States. McBeth, 1938, Raski and Hart, 1953, and Gerdemann and Linford, 1953, found the species in pastures, lawns and roadsides primarily associated with white clover, *Trifolium repens* L. In Europe the nematode is widely distributed in Germany (Goffart, 1932, 1944), the Netherlands (Oostenbrink, 1949, 1954, 1951) and England (Franklin, 1939, 1940, 1945, 1951; Fenwick and Franklin, 1951). Recently *H. trifolii* has been observed on white clover in 2 localities in North Carolina.

An overlapping in the host plants which are attacked by *H. glycines* and *H. trifolii* has been reported. Both species reproduce on *Phaseolus vulgaris* L., *Lespedeza stipulacea* Maxim. and *Vicia sativa* L. Moreover *H. glycines* and *H. trifolii* belong to the group of closely related *Heterodera* species with lemon-shaped cysts which are very similar morphologically. The investigation was undertaken to compare cysts and their contents and to determine whether morphological characteristics could be used to differentiate the 2 species. A preliminary report on the morphological comparison of *H. glycines* and *H. trifolii* has been given (Hirschmann, 1956).

*Contribution from Plant Pathology, North Carolina Agricultural Experiment Station, Raleigh, North Carolina. Published with the approval of the Director of Research as Paper No. 724 of the Journal Series.

MATERIALS AND METHODS.—The nematode material used in this study was obtained from greenhouse cultures of *H. glycines* on soybean, *Glycine max* (L.), and of *H. trifolii* on white clover, *Trifolium repens* L. Cysts of both species were collected originally from infested areas in North Carolina. The technique developed by Fenwick and Franklin, 1942, was used in making measurements.

COMPARISON OF CYST CHARACTERS.—*H. glycines* as well as *H. trifolii* form lemon-shaped cysts with a prominent vulva posteriorly (Fig. 1). In both species dark knob-like projections (brown knobs) can be found around the vulval aperture on the inside of the cyst wall. They appear to be of the same material as the wall. The young females of *H. glycines* and *H. trifolii* are at first white, then pale yellow and turn into dead dark-brown cysts of similar color. The newly developed females are coated with a white "subcrystalline layer" which persists on the brown cysts for some time. A gelatinous egg sac, measuring from ⅓ of the body size to the full body size in larger cysts, is attached to the vulva of both species (Fig. 1). The egg sac is formed shortly after the female has ruptured the root cortex. The number of eggs usually deposited into it varies from a few to about 200 in each species. The function of the gelatinous material seems to be protective as in *Meloidogyne*.

The length (excluding the neck) and the breadth of 100 cysts of each species were measured from camera lucida outline drawings to determine differences, if any, in the size or shape of the cysts in the 2 species. The

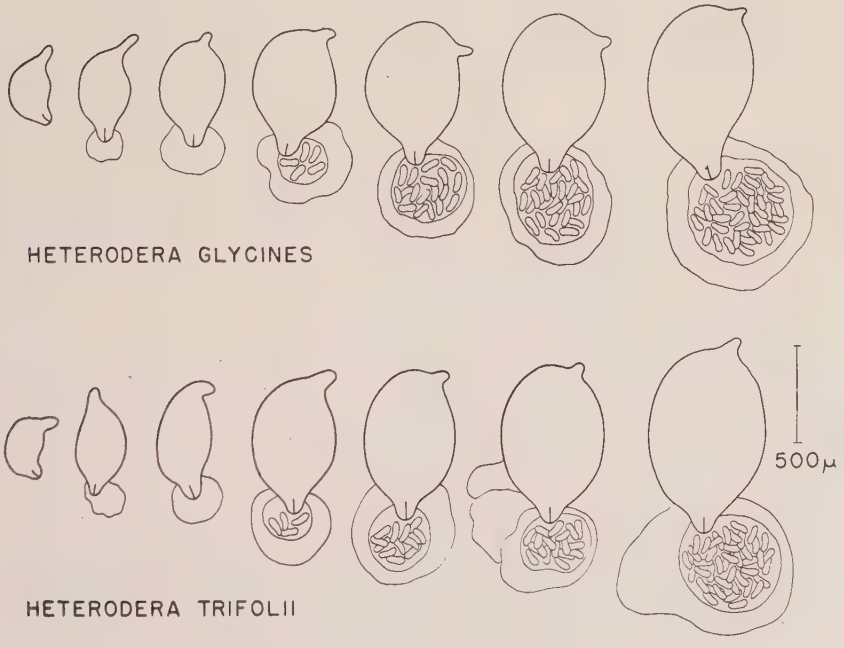

Figure 1. Outline diagrams to illustrate the shape and size ranges of cysts, the form of the egg sac and the extrusion of eggs in *H. glycines* and *H. trifolii*.

data were analysed statistically and are given in Table 1 a and b. Differences in the length/breadth relationships (length/breadth ratio, regression coefficient length/breadth, regression coefficient breadth/length) are not great enough to be useful in separating *H. glycines* and *H. trifolii.*

The structure of the cyst cone and the distance from vulva to anus, two cyst features which have been reported to be of assistance in differentiating other *Heterodera* species (Oostenbrink and Ouden, 1954; Granek, 1955) were also studied (Table 1 c, Fig. 2A, B). No marked differences in the size of the transparent lip tops of the cone, in the length of the vulva split or in the distance from vulva to anus were found.

The outer layer of the cyst wall exhibits the same rugose pattern of zig-zag lines in both species. The clearness of the pattern may possibly be influenced by the degree of desiccation of the cyst, its age or size. In young small cysts (Fig. 3A, C) the pattern is very distinct and the zig-zag lines are close together. In larger cysts the lines are wider apart and somewhat fainter (Fig. 3B, D). Aside from the variation due to size, age or degree of desiccation there is no difference in the zig-zag pattern of the cysts in the 2 species. The punctation of the inner layer of the cyst wall is arranged irregularly in both species, but seems to be somewhat finer in *H. trifolii* than in *H. glycines.*

Comparison of the Eggs.—The number of eggs per cyst ranges from about 25 to 400 in both species. Measurements of the length and breadth of 300 eggs (using 10 eggs from each of 30 cysts) in each species showed no significant difference in size, shape or length/breadth ratio of the eggs (Table 2).

Comparison of the Second Stage Larvae.—The most significant morphological differences between the 2 species were present in the second stage larvae (Figs. 4, 5, 6, 7, 8). Consistent characters for separating the 2 species are body length, shape and size of the stylet, distance of the dorsal gland orifice behind the stylet knobs, shape and length of the tail, and length of the tail terminal. The stylet of *H. glycines* (Figs. 6A, B; 7 A, B, C) is shorter and appears somewhat stouter than that of *H. trifolii* (Figs. 6 C, D; 7 D, E, F). While in *H. glycines* the basal knobs are distinctly separated and much broader than high, they are closer together in *H. trifolii,* which gives them a more rounded appearance posteriorly. They are almost as high as broad and appear to be hollowed out anteriorly. The apparent shape of the basal knobs changes somewhat with the position of the stylet, as illustrated in Fig. 7, where stylets of *H. glycines* in the upper row are compared with those of *H. trifolii* in the lower row in lateral, dorsal and ventral view. The basic form, however, remains the same in each species. The dorsal esophageal gland orifice opens far behind the stylet knobs in *H. trifolii* (Fig. 7 D, E, F), whereas it is rather close in *H. glycines* (Fig. 7 A, B, C). In comparing the tails and tail terminals of the two species (Fig. 8) their different shape and size is apparent. *H. glycines* (Fig. 8 A, B, C) has a shorter, plumper tail with a more bluntly rounded terminus and a shorter tail terminal than *H. trifolii* (Fig. 8 D, E, F). The location of the phasmids was not used as a taxonomic character, since they often varied in position as shown by the tails in ventral view (Fig. 8 B, E).

Measurements of *H. glycines* and *H. trifolii* larval characters are compared in Table 3. The statistical analysis based on measurements of 5

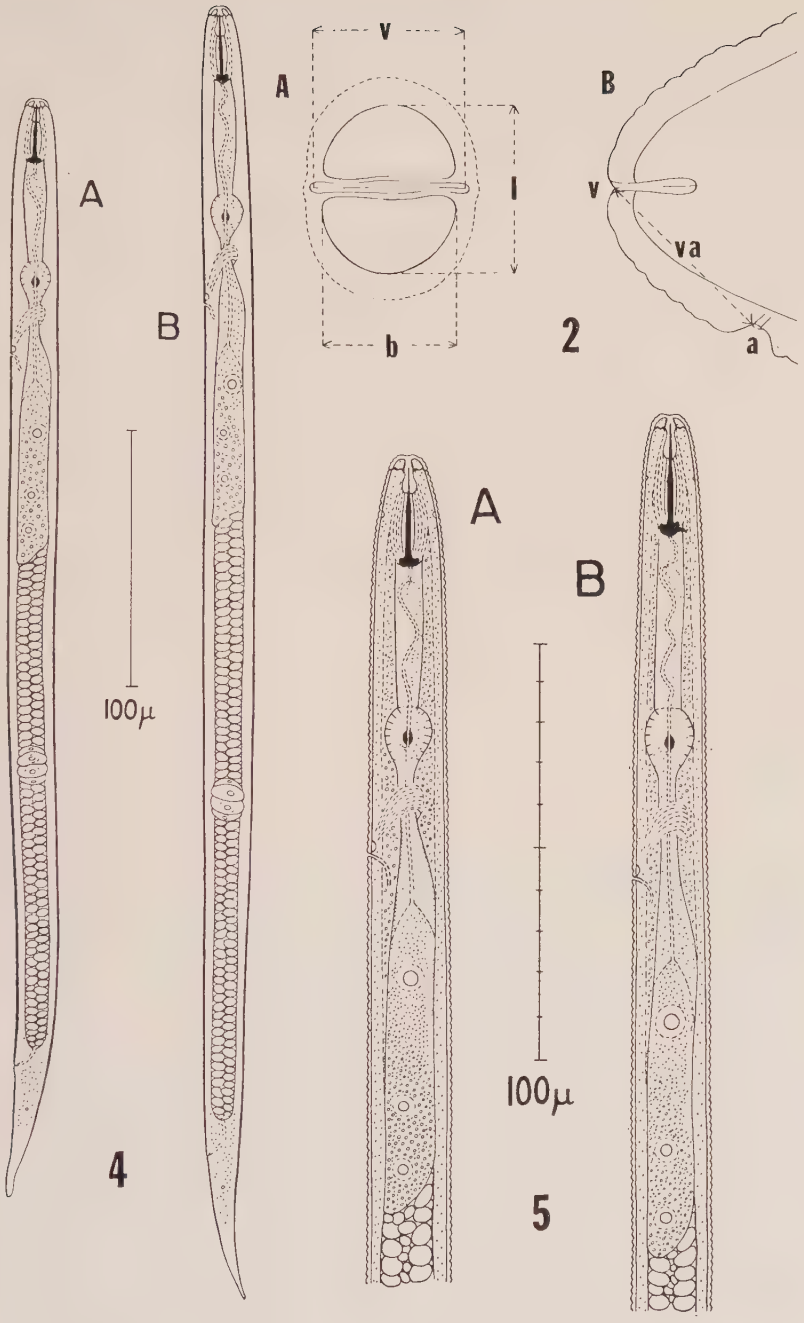

Figure 2. Diagram of the cyst cone. A = top view; B = lateral view; v = vulva split, l = maximum length of light patches; b = maximum breadth of light patches; va = distance from vulva to anus; a = anus.

Figure 4. Second stage larvae, lateral view. A = *Heterodera glycines;* B = *Heterodera trifolii.*

Figure 5. Second stage larvae, anterior portion, lateral. A = *H. glycines;* B = *H. trifolii.*

TABLE 1.—Comparison of cyst characters of *Heterodera glycines* and *Heterodera trifolii*

(a) Size ranges (in microns) and length/breadth ratios*

Species	Maxima		Minima		Range		Ratio
	Length	Breadth	Length	Breadth	Length	Breadth	Length/Breadth
H. glycines	920	560	340	200	580	360	2.05 to 1.19
H. trifolii	995	615	310	190	685	425	2.40 to 1.32

* based on 100 cysts of each species (without neck).

(b) Regression coefficients calculated from length and breadth measurements

Species	Number of cysts measured	Regression coefficient length/breadth	Mean coefficient (%)	Regression coefficient breadth/length	Mean coefficient (%)
H. glycines	100	1.3084	99.7	0.6482	103.6
H. trifolii	100	1.3173	100.3	0.6028	96.4
	Total 200	Mean 1.3129	100.0	0.6255	100.0
		S.E. ±0.0625	±4.8	±0.0296	±4.7
	Sign diff.		±13.1		±13.2

(c) Measurements of the characters of the cyst cone (in microns) (see also Fig. 2)**

Species	Maximum length of light patches (l)				Maximum breadth of light patches (b)			
	Maxima	Minima	Range	Mean	Maxima	Minima	Range	Mean
H. glycines	65	37	28	53.7	48	33	15	40.5
H. trifolii	60	45	15	54.6	49	31	18	40.2

Species	Length of Vulva split (v)				Distance from Vulva to anus (va)			
	Maxima	Minima	Range	Mean	Maxima	Minima	Range	Mean
H. glycines	56	43	13	49.7	77	59	18	67.5
H. trifolii	58	39	19	47.8	68	54	14	61.4

** based on 15 measurements for each character and species.

larvae from each of 30 cysts for each species shows no overlapping in the means of the various characters. Moreover, the range of stylet length and gland orifice do not overlap. This indicates that the stylet length is a good character for separating the two species. The gland orifice is less satisfactory because of the difficulties in exact measurement.

OCCURRENCE OF MALES.—Although Franklin and McBeth described males, no males were observed in *H. trifolii*. This agrees with the work of Raski and Hart, 1953, and Gerdemann and Linford, 1953. The body length varies considerably as found in males of other species of *Heterodera* (Table 4). The lip region bears 5 annules, the cephalic framework is heavily sclerotized (Fig. 9 A, B). The dorsal gland orifice opens a short distance behind the rounded knobs of the stout stylet. The spicules are the bidentate type, the phasmids are very minute (Fig. 9 C).

During the detailed morphological study of the males, the presence of an apparently hitherto unrecorded structure was noted in the head region. It is situated 5 to 7 annules posteriorly to the constriction of the lip region.

The structure is bandlike and since it can be seen in lateral and ventral view seems to extend around the head region. In optical section it appears more or less biconvex and is situated between the cuticle and hypodermal layer. The structure is also present in the same position, but somewhat less pronounced in the second stage larvae of both *H. glycines* and *H. trifolii*. It might be similar to the "hemizonid" which is located 3 to 8 annules anteriorly to the excretory pore in the males of *H. glycines* (Fig. 9 A).

DETERMINATION OF THE MOST EFFICIENT SAMPLE SIZE.*—When this investigation was initiated the sample size for the number of cysts to be used and the number of larvae per cyst to be measured was not known, since no similar experiments were available in the literature. The sample of 5 larvae from each of 30 cysts was entirely based on guess work. As a guide for similar investigations the most efficient sample size based upon the above investigations with 30 cysts and 5 larvae per cyst was determined. The body length of *H. trifolii* larvae was selected arbitrarily for this study.

The first step was to determine how many larvae per cyst should be measured. To resolve this, the variances for mean larval length based on different numbers of cysts from 5 to 15 and different numbers of larvae from 1 to 5 were computed using

$$V = \frac{\sigma c^2}{n} + \frac{\sigma L^2}{mn} = \frac{125}{n} + \frac{250}{mn},$$

where n = the number of cysts, m = the number of larvae per cyst, δ_c^2 = the variability between cysts, σL^2 = the variability between larvae within

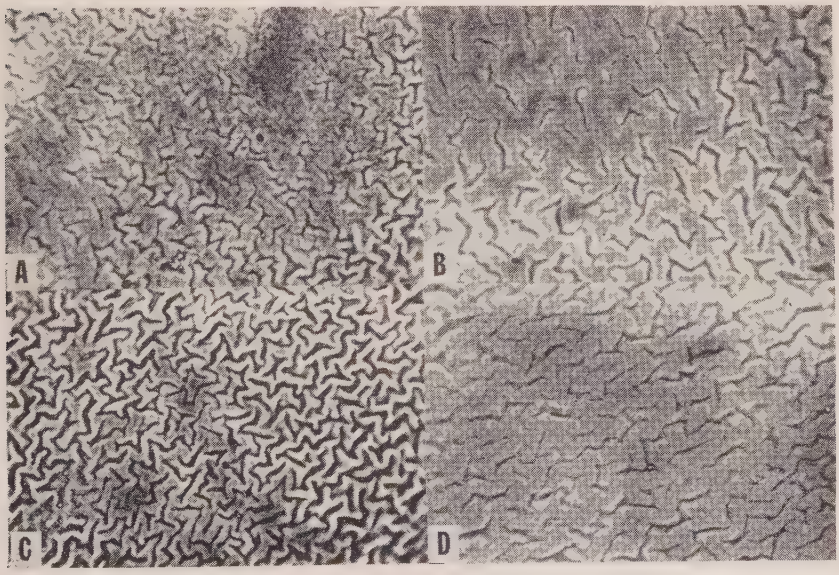

Figure 3. Photographs of cyst patterns. A = H. *glycines*, small cyst; B = H, *glycines*, large cyst; C = H. *trifolii*, small cyst; D = H. *trifolii*, large cyst. All the same magnification.

TABLE 2.—Comparison of eggs of *H. glycines* and *H. trifolii* (in microns)*

Species	Maxima		Minima		Range		Ratio
	Length	Breadth	Length	Breadth	Length	Breadth	Length Breadth
H. glycines	118	45	80	30	38	15	2.59
H. trifolii	120	50	92	39	28	11	2.39

* based on 300 eggs for each species (10 eggs from each of 30 cysts).

TABLE 3.—Comparison of larval characters of *Heterodera glycines* and *Heterodera trifolii* (in microns)

Character	*Heterodera glycines**		*Heterodera trifolii**	
	Range	Mean**	Range	Mean**
Body length	375.0 — 490.0	439.6 ± 6.7	443.0 — 547.0	496.6 ± 6.7
Stylet length	22.0 — 24.0	23.0 ± 0.1	25.5 — 29.5	27.5 ± 0.3
Gland orifice	3.0 — 5.2	4.0 ± 0.2	5.6 — 9.0	7.3 ± 0.2
Tail length	42.0 — 59.4	50.4 ± 1.0	51.0 — 71.1	60.3 ± 1.1
Tail terminal length	20.0 — 33.0	26.6 ± 0.7	24.2 — 39.6	30.7 ± 0.9

* Measurements based on 150 larvae (5 larvae from each of 30 cysts).
** 99% confidence interval.

TABLE 4.—Measurements of the males of *Heterodera glycines* (in microns)*

Character	Range	Mean**
Body length	1035.0 — 1400.0	1265.0 ± 50.9
Breadth	26.8 — 30.6	28.6 ± 0.5
Stylet length	25.5 — 28.4	26.9 ± 0.4
Gland orifice	3.2 — 4.2	3.9 ± 0.1
Esophagus	127.2 — 176.0	154.6 ± 6.1
Tail length	3.7 — 8.4	5.3 ± 0.5
Spicula	33.5 — 36.8	34.3 ± 1.2
Gubernaculum	9.9 — 12.5	11.4 ± 1.1

* Sample consisting of 20 males.
** 95% confidence interval.

TABLE 5.—Variances for mean larval length (in μ^2) in *Heterodera trifolii* based on respective numbers of cysts and larvae per cyst

Larvae per cyst (m)	Number of cysts (n)										
	5	6	7	8	9	10	11	12	13	14	15
1	75.0	62.5	53.6	46.9	41.7	37.5	34.1	31.2	28.8	26.8	25.0
	45.9*	42.9	30.7	23.0	18.2	19.8	17.2	25.8	37.4	32.5	28.7
2	50.0	41.6	35.8	31.2	27.8	25.0	22.8	20.8	19.2	17.8	16.6
	2.7	10.3	11.3	11.3	8.8	11.3	14.1	14.3	22.5	19.4	16.8
3	41.7	34.7	29.8	26.0	23.2	20.8	19.0	17.3	16.0	14.8	13.9
	12.9	15.2	10.9	11.0	6.5	6.8	9.2	10.7	14.9	12.8	11.2
4	37.5	31.2	26.8	23.4	20.8	18.7	17.1	15.6	14.4	13.4	12.5
	9.9	12.7	9.3	9.6	5.8	7.2	9.5	13.3	12.5	10.8	10.8
5	35.0	29.1	25.0	21.9	19.5	17.5	15.9	14.6	13.4	12.5	11.6
	8.6	12.3	9.1	9.3	6.1	8.8	10.6	13.9	13.0	11.6	11.7

* Variances computed from actual samples.

TABLE 6.—Estimated numbers of cysts and larvae required for precision of mean larval length (microns) equivalent to that obtained with 30 cysts and 5 larvae ($V_{\bar{x}} = 5.83$)

	Number of larvae per cyst (m)				
	1	2	3	4	5
Number of cysts (n)	64.3	42.9	35.7	32.2	30.0
95% confidence limits for number of cysts	91.4, 36.9	63.7, 21.7	54.6, 16.4	50.0, 13.9	49.8, 16.5

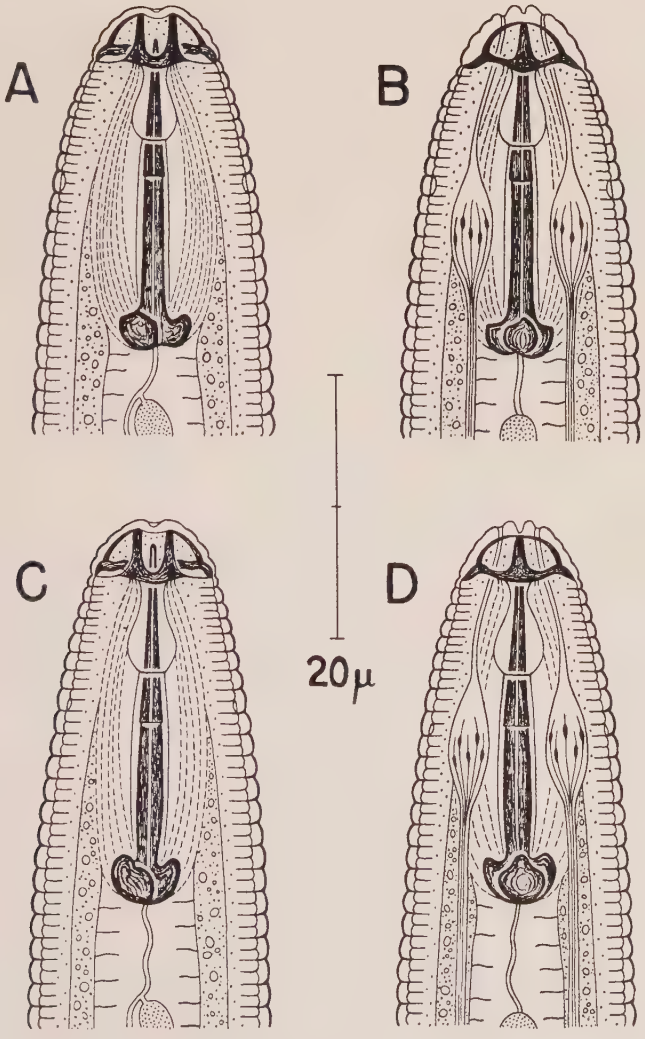

Figure 6. Second stage larvae, heads. A = H. *glycines*, lateral; B = *H, glycines*, dorsal; C = *H. trifolii*, lateral; D = *H. trifolii*, dorsal.

cysts. The variance components used in the computation were estimated from the data obtained from the sample of 30 cysts and 5 larvae per cyst. Table 5 indicates that measuring more than 3 larvae from each cyst does not materially reduce the variances of the overall means. It was further noted (data not shown) that taking more than 3 larvae per cyst does not improve the precision of mean larval length for each cyst.

The second step was to determine how many cysts one should sample in order to achieve a specified variance, say V, of the sample mean. The formula used was

$$n = \frac{1}{V}\left[\sigma c^2 + \frac{\sigma L^2}{m}\right]$$

The appropriate number of cysts has been computed for $V = 5.83$, $\delta_c^2 = 125$, $\sigma L^2 = 250$, the values observed for the actual samples of 30 cysts and 5 larvae per cyst. The results are given in Table 6. Examination of this table shows that the same precision is attained with 1 larva from each of 64 cysts, 2 larvae from each of 43 cysts and so on, as with 5 larvae and 30 cysts. According to the conclusion reached in estimating the numbers of larvae we need only to have measured 3 larvae from each of 36 cysts. Since the measuring of the different larval characters requires more time than the opening of the cysts, the same precision would have been attained with less effort. The confidence limits for the estimates of the number of cysts to sample in Table 6 were computed using an approximate method according to Wilson and Hilferty (cf. M. G. Kendall, 1946).

Summary

A detailed comparison of cysts, eggs and second stage larvae of the soybean cyst nematode, *Heterodera glycines,* and the clover cyst nematode, *H. trifolii,* was made. No differences were found in the characters of the lemon-shaped cysts. In both species the young females are coated with a "subcrystalline layer," and pass through a yellow color phase, when changing from white to brown. The cysts are dark brown in color and have brown knobs around the vulval aperture. There is no detectable difference in the shape and the size of the gelatinous egg sac, or in the number of eggs extruded into it. Length/breadth measurements were taken on 100 cysts of each species. The length/breadth ratio and the regression coefficients for length/breadth and breadth/length show no significant differences. The size of the transparent lip tops of the vulva cone, the length of the vulva split and the distance from vulva to anus differ only slightly. The cyst wall exhibits the same pattern of zig-zag lines in the two species.

Length/breadth measurements of 300 eggs in each species showed no dissimilarity in size, shape or length/breadth ratio of the eggs.

Consistent morphological differences were found in the second stage larvae of the two species. Characters which differ considerably are body length, shape and size of the stylet, distance of the dorsal gland orifice behind the stylet knobs, shape and length of the tail, as well as the length of the tail terminal. Measurements of these characters were taken on 5 larvae from each of 30 cysts in each species. There was no overlapping in the means of the various characters.

*The author is greatly indebted to Dr. B. D. Tikkiwal, Jaipur, India, for assistance in the statistical analysis.

No males were present in *H. trifolii,* while they were abundant in *H. glycines.* In the head region of the male a structure was observed which might be similar to the "hemizonid" located anteriorly to the excretory pore.

As a guide for similar investigations the most efficient sample size for measurements of the different larval characters was determined based on the results obtained with 30 cysts and 5 larvae per cyst.

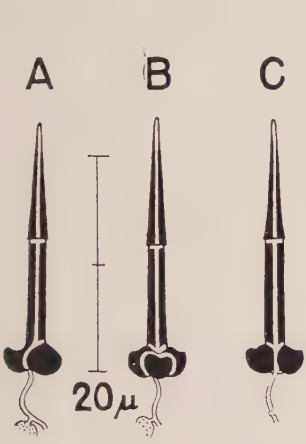

Figure 7. Second stage larvae, stylets. A = *H. glycines,* lateral; B = *H. glycines,* dorsal; C = *H. glycines,* ventral; D = *H. trifolii,* lateral; E = *H. trifolii,* dorsal; F = *H. trifolii,* ventral.

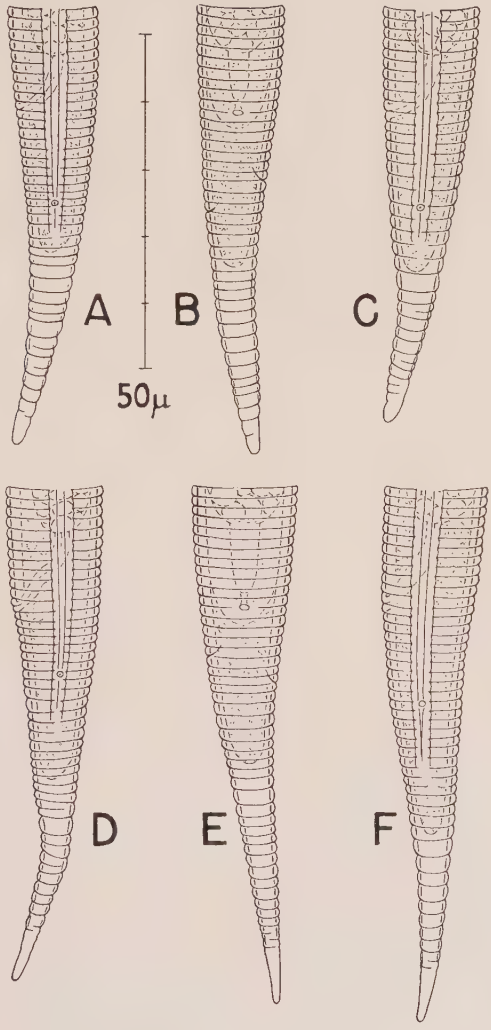

Figure 8. Second stage larvae, tails. A, C = *H. glycines,* lateral; B = *H. glycines,* ventral; D, F = *H. trifolii,* lateral; E = *H. trifolii,* ventral.

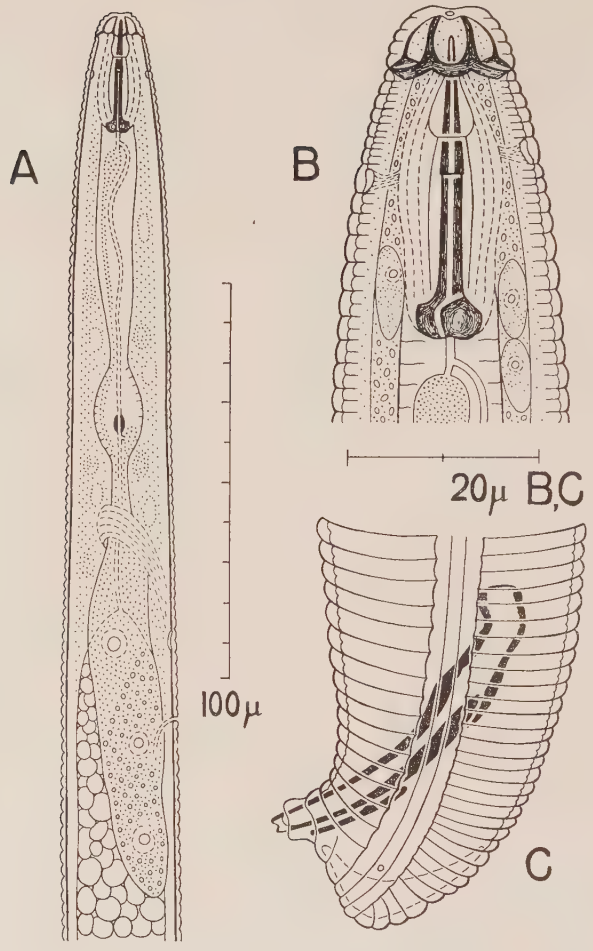

Figure 9. *Heterodera glycines*, male, lateral. A = Esophagus; B = Head; C = Tail.

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Tylenchorhynchus lenorus, n. sp. (Nematoda: Tylenchida), Associated with the Roots of Wheat*

Georgiana L. Brown**

Roots of wheat collected near Lake Lenore, Saskatchewan, by R. C. Russell of the Botany and Plant Pathology Division, Science Service, Saskatoon, harbored a new species of nematode belonging to the genus *Tylenchorhynchus,* herein named *T. lenorus,* n. sp. This species was found in a mixed population consisting of *T. leptus* Allen, 1955, and probably 4 other new species of *Tylenchorhynchus.* The field from which the wheat plants were taken had been broken only the previous year from undisturbed grassland. The description of *Tylenchorhynchus lenorus,* n. sp., follows (Figure 1):

Dimensions.—10 ♀♀: length, .63-.78 mm.; a = 22-29; b = 5.2-6.7; c = 12-15; v = 52-58%; stylet 18-20 microns long. 10 ♂♂; length, .56-.68; a = 26-34; b = 4.8-5.7; c = 12-15; stylet 18-20 microns long.

Female (holotype): length, .66 mm; a = 28; b = 5.2; c = 12; v = 52%.

Body cylindrical, tapering at both ends, slightly curved ventrally when relaxed by gentle heat. Lip region well set off from body contour, bearing 6 annules. Labial framework lightly sclerotized. Stylet 19μ long, with rounded knobs. Orifice of dorsal esophageal gland 2μ behind stylet base. Median esophageal bulb slightly ovoid; basal bulb well developed, joined to intestine by a rounded cardia. Excretory pore opening at level of middle of basal esophageal bulb. Vulva near middle of body. Ovaries two, outstretched. Spermatheca present. Tail conoid-obtuse, with annulation of cuticle not extending around terminus. Cuticle bearing transverse and longitudinal striae. About 24 longitudinal striae on middle of body, dividing cuticle into squares. Lateral field with 6 incisures beginning just posterior to basal esophageal bulb and decreasing to 4 incisures near tip of tail. Phasmids prominent, opening at middle of tail.

Male (allotype): length, .66 mm; a = 30; b = 5.4; c = 13; stylet 20 microns long.

Similar to female. Testes single, outstretched. Spicula typical tylenchoid form. Gubernaculum simple, slightly curved. Bursa enveloping tail. Phasmids opening at middle of tail.

Diagnosis.—*Tylenchorhynchus lenorus* keys to *T. ornatus* according to Allen, 1955, but differs from it in having a set-off lip region, a conoid-obtuse tail, and fewer longitudinal striae. *T. lenorus* differs from *T. quadrifer* in having fewer longitudinal striae and from *T. tessalatus* in being smaller in size and in having no annulations around terminus of tail.

Type Locality.—A field 4 miles west of the town of Lake Lenore, Saskatchewan.

Habitat.—Soil around roots of wheat.

Type Specimens.—(Holotype) female collected August 1955 by R. C. Russell, Collection No. 392; (allotype) male, same data as holotype; 40 paratypes in Canadian National Collection of Nematodes.

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*From Nematode Investigations Section, Entomology Laboratory, Ottawa. Contribution No. 3417, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.

**Associate Nematologist.

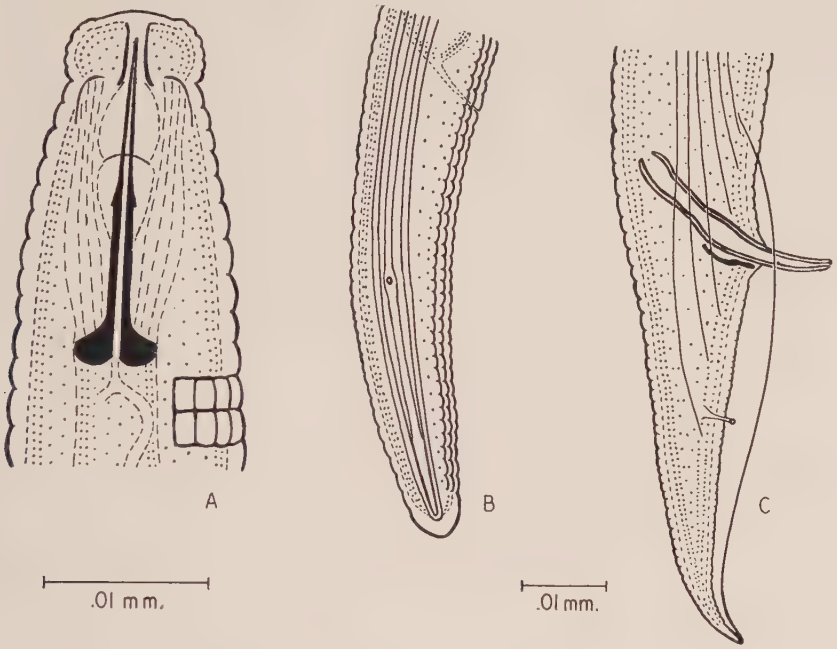

Fig. 1. *Tylenchorhynchus lenorus* n. sp. A. Head. B. Female tail. C. Male tail.

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Monogenetic trematodes of Gulf of Mexico Fishes. Part XI. The family Microcotylidae Taschenberg, 1879. (Continued)*

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Virginia Fisheries Laboratory, Gloucester Point, Virginia

In this, the eleventh installment of the present series treating the monogeneids of Gulf of Mexico fishes, the subfamily Axininae Monticelli, 1903 is revised and emended. Several new or previously described species belonging to the genera *Axinoides* Yamaguti, 1938 *diag. emend., Cemocotyle* Sproston, 1946 and *Heteraxine* Yamaguti, 1938 are described and/or discussed. The organization and methods are the same as for preceding installments.

Suborder Polyopisthocotylea Odhner, 1912
Superfamily Diclidophoroidea Price, 1936
Family Microcotylidae Taschenberg, 1879
Subfamily Axininae Monticelli, 1903, *DIAG. EMEND.*

Diagnosis: Microcotylidae. Body elongate, flattened dorso-ventrally, widened posteriorly. Growth of posterior portion of body asymmetrical so

*Contribution from the Zoology Department and Oceanographic Institute of Florida State University, Tallahassee, Florida.

that the embryonically posterior end is usually lateral. Opisthaptor, therefore, an asymmetrical cotylophore. Clamp rows either entirely on one margin and arranged so that the two rows are nearly tandem or, if on both sides one row is much shorter than the other and contains fewer clamps. Clamps somewhat variable in shape, but the basic sclerite pattern is like that of Microcotylinae. Anchors usually retained by adult, their position serving to indicate the embryologically posterior end. Anchors different from those of other groups, much like those on the post-larval stage of *Microcotyle*. Cuticle often papillated. Genital atrium armed or not. Cirrus armed or not. Vaginal pore dorsal or marginal, may be armed with a sclerotized papilla.

Type Genus: *Axine* Abildgaard, 1794.

Discussion. The subfamily Axininae differs from the subfamily Microcotylinae as conceived in the present series in the following characteristics: (1) cotylophore laterally asymmetrical, (2) embryonically posterior end lateral in mature specimens, (3) anchors retained by adults, (4) general triangular body shape. However, general similarities of the arrangement of internal organs, anchor shape and basic clamp sclerites indicate that these subfamilies are related. Also, some relationship of this group with members of the family Discocotylidae may be indicated by the straight shafts and lunate ends of the medial anchors. Hargis (1954) erroneously listed Axiniinae (a misspelling) as a new subfamily not realizing that it was first used by Monticelli (1903) (not Monticelli, 1892 as given by Chauhan, 1953) and again by Nicoll (1915). Even though this group was rejected by subsequent workers and combined with Microcotylinae, it is felt that the differences between these taxa are too great to be ignored. New evidence necessitates its revival and emendation.

The genera included in Axininae are: *Axine* Abildgaard, 1794, *Axinoides* Yamaguti, 1938, *diag. emend.*, *Heteraxine* Yamaguti, 1938, *sensu* Sproston, 1946, *Cemocotyle* Sproston, 1946 and *Lintaxine* Sproston, 1946. The last genus needs additional study in order to clarify its taxonomic position. *Cemocotyle* which is very similar to *Heteraxine* and may be congeneric with it, appears to be much like members of the sub family Microcotylinae. It may later be shown that this or a similar group was probably the phylogenetic link between the two subfamilies.

Genus *Axinoides* Yamaguti, 1938, Diag. Emend.

Diagnosis: Axininae. Body elongate, flattened dorso-ventrally, widened posteriorly to a somewhat triangular shape. Cotylophore with similar clamps in two, nearly tandem rows which face obliquely laterad. Two dissimilar pairs of anchors usually present. Cirrus muscular, usually armed with terminal papillae which are directed posteriorly when cirrus is everted. Genital atrium not armed. Vagina may be supramarginal to middorsal, armed with a cuticularized papillae.

Type Species: *Axinoides tylosuri* Yamaguti, 1938.

Discussion: *Axinoides* is very similar to *Axine* Abildgaard, 1794 from which it differs in the following respects: (1) genital atrium not armed, (2) cirrus armanent consists of cuticularized papillae, (3) variable position of vagina.

Both of these genera should be studied and re-evaluated. Their differential characteristics may actually be only of subgeneric importance.

Axinoides gracilis (Linton, 1940) Sproston, 1946

Host: *Tylosurus marinus* (Walbaum), Needlefish, a nerito-pelagic marine belonid.

Location: Gills.

Locality: Alligator Harbor, Florida.

Previously reported host and locality: *Tylosurus marina* [=*Strongylura marina*] from Woods Hole, Massachusetts.

Number studied: 53.

Discussion: Even though the type slides were not available for this study, it appears evident that the worms in the present collection are conspecific with Linton's (1940) species. The following additional information was obtained: Cephalic glands present. The usual 2 pairs of anchors, which Linton did not figure, are present on the opisthaptor. The series of specimens in this collection demonstrated that the vaginal pore and papillate sclerite may vary in position from mid-dorsal to slightly supramarginal. This discovery destroys the value of Sproston's (1946) distinction between *Axine* and *Axinoides* on the basis of vaginal pore position and necessitates the emendations made above. Cirrus muscular and apparently not armed. This species badly needs redescription. *A. gracilis* is closely related to *A. raphidoma* n. sp.

Axinoides raphidoma n. sp., (Figs. 1-4).

Host: *Tylosurus raphidoma* (Ranzani), Hound Fish, a nerito-pelagic marine belonid.

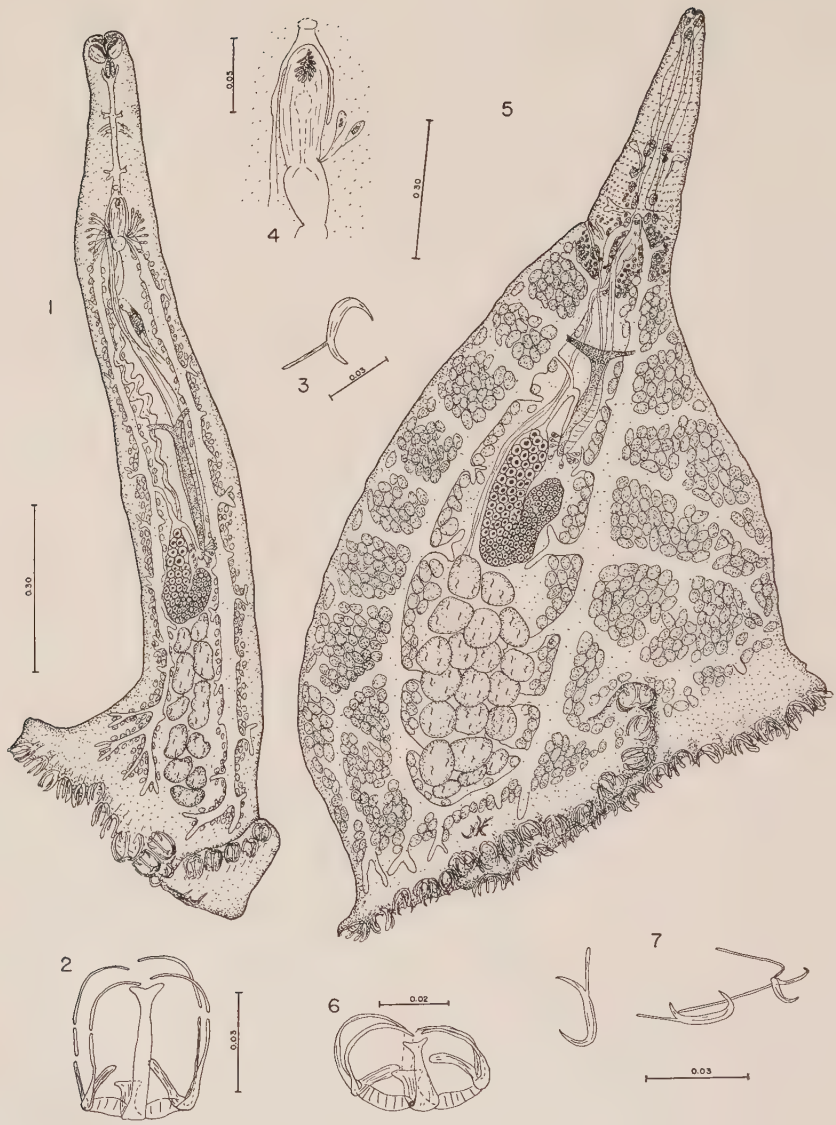
Location: Gills.

Locality: Alligator Harbor, Franklin Co., Florida.

Number studied and measured: 1.

Holotype USNM Coll. No. 38156.

Description: Body elongate, sides converging anteriorly, 1.6 long by 0.3 wide, anterior end bluntly rounded and cleft longitudinally into two lobes, body widened posteriorly. Cuticle thin, with delicate transverse striae. Prohaptor a pair of hemispherical buccal suckers, 0.039 in diameter, posterolateral in bussal funnel. Opisthaptor an asymmetrical, truncate cotylophore, 0.050 long, obliquely oriented, long end to left, armed by two rows of clamps and 2 pairs of anchors; anchors situated medically on cotylophore indicate the embroyonically posterior end; the two clamp rows are tandem, end to end, giving the appearance of a single row of 17 clamps. Clamps slightly pedunculated, 0.053 long by 0.044 wide; sclerites tpyically microcotylid in nature, delicate, ventral loop incomplete medially and laterally, in 4 parts, dorsal loop elements fairly stout, middle loop interrupted laterally and medially into 4 or 5 parts, center piece shorter ventrally than dorsally and widened at both ends. Anchors delicate, of two types; lateral pair shorter, 0.052 long, with delicate shafts and lunate hooks; medial pair longer, somewhat discocotylid in shape, 0.041 long, with more delicate shafts and smaller lunate hooks. Mouth subterminal; short buccal funnel. Pharynx delicate, cylindrical 0.027 long by 0.019 wide; esophagus long, laterally ramified. Gut bifurcated, crura ramified, rami branched, crura not confluent posteriorly. Testes follicular, about 11 in number, between intestinal crura posequatorially; vas deferens sinuous, running anteriorly to join seminal vesicle at base of cirrus. Cirrus muscular, eversible and

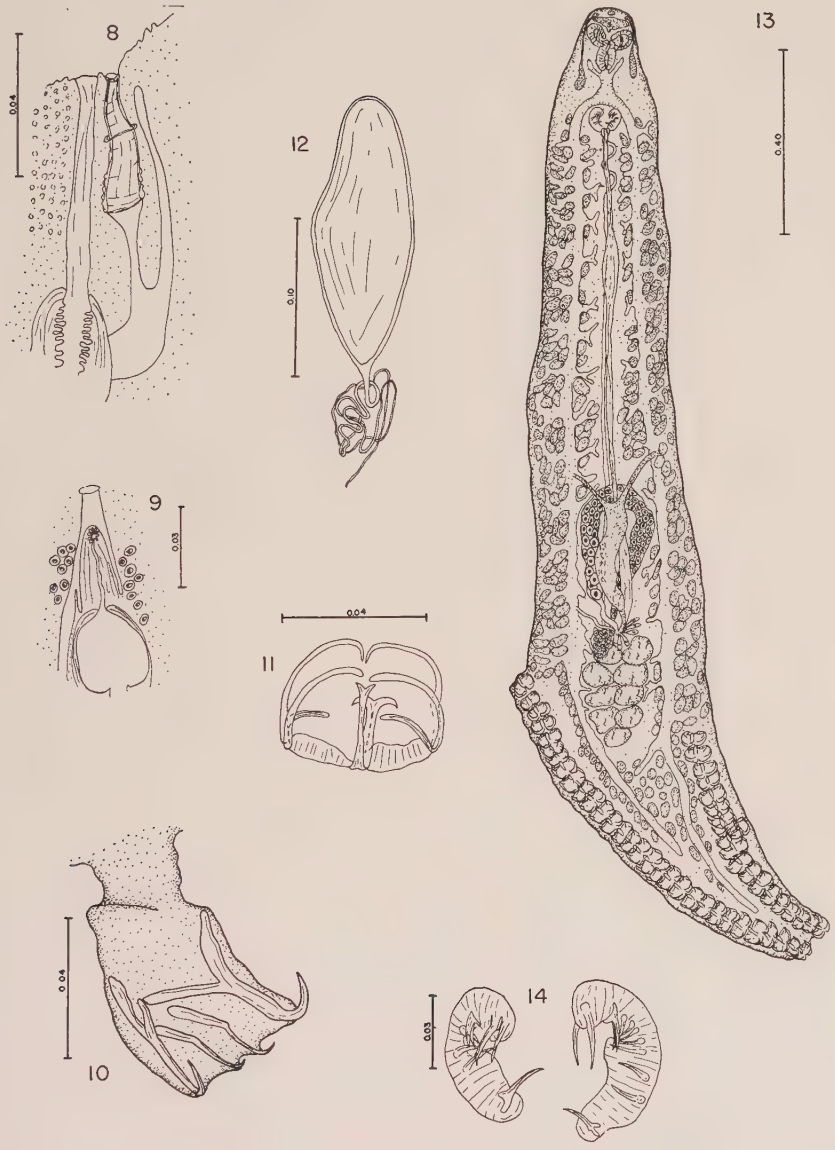


Axinoides raphidoma n. sp.

1. Whole mount, vertical view.
2. Clamp, ventral view.
3. Anchor.
4. Enlargement of cirrus and genital atrium.

Axinoides truncatus n. sp.

5. Whole mount, ventral view.
6. Clamp, ventral view.
7. Anchors.



Axinoides truncatus n. sp.

8. Enlargement of vaginal complex showing vaginal pore and sclerotized vaginal cone with their ducts.
9. Cirrus, genital atrium and seminal vesticle.

Heteraxine xanthophilus n. sp.

10. Anchors of embryo.
11. Clamp, ventral view.
12. Egg, *in utero*.
13. Whole mount, ventral view.
14. Genital atrium arament, showing muscular pieces and spines.

protrusible, 0.070 long by 0.034 wide bearing muscular papillae in anterior end of lumen which point outwardly when cirrus is everted. Spherical body at base of cirrus may act as both seminal vesicle and prostate reservoir. Prostate gland cells at base of cirrus. Genital pore midventral to esophagus at one-fourth body level, opening into atrium. Ovary pretesticular, tubular, curving from left to right; oviduct wide, coursing medially. Ootype fusiform, dorsal to vitelline reservoir; uterus slightly curved entering genital atrium anteriorly from right side. Genito-intestinal canal joining right crus with oviduct. Vaginal pore nearly middorsal, armed with a sclerotized papilla; vaginal duct extending obliquely to the left, walls of distal portion of vaginal duct muscular and thrown up into folds, posterior portion of vaginal ducts not observed. Mehlis' gland present. Vitellaria follicular, near intestinal crura, extending from level slightly posterior to cirrus to posterior ends of crura; transverse vitelloducts fusing medially to form long Y-shaped vitelline reservoir. Egg not observed. Brain dorsal to medial portion of esophagus.

DISCUSSION: *Axinoides raphidoma* n. sp. appears to be closely related to *A. tylosuri* Yamaguti, 1938 and *A. aberrans* (Goto, 1894) Sproston, 1946 from which it differs in the following respects: (1) smaller body size, (2) fewer testes, (3) fewer clamps, (4) dorsal loops of clamps stouter and straighter, (5) hosts.

Axinoides truncatus n. sp., (Figs. 5-9)

HOST: *Tylosurus raphidoma* (Ranzani), Hound Fish, a nerito-pelagic marine belonid.

LOCATION: Gills.

LOCALITY: Alligator Harbor, Franklin Co., Florida.

Number studied and measured: 2.

HOLOTYPE USNM Helm. Coll. No. 38157.

DESCRIPTION: Body subtriangular in shape, flattened dorsal-ventrally, 2.1 (2.0-2.2) long by 1.0 (0.8-1.1) wide, narrow anteriorly to a bilobed, rounded end, widened posteriorly to a very broad, obliquely truncate end. Cuticle fairly thick with transverse rows of regularly arranged cuticular papillae. Prohaptor a pair of hemispherical buccal suckers, 0.022 (0.020-0.024) in diameter, in lateral walls of buccal funnel; several pairs of head organs connected by long ducts to 5 pairs of posteriorly located cephalic glands. Opisthaptor an obliquely placed cotylophore whose longer side is dextral, 1.2 (1.0-1.3) long, armed posteriorly with 2 rows of similar clamps, right row with 15-16 clamps and left row with 10-13; clamp rows tending toward a tandem position but still overlapping medially; embryonically posterior end indicated by the presence of 2 pairs of anchors about one-third from the actual posterior end on right. Clamps typically microcotylid in structure, larger medially than terminally, about 0.056 (0.036-0.075) long by 0.041 (0.027-0.055) wide; ventral loop incomplete medially but not laterally, dorsal loop elements present, middle loop incomplete medially but not laterally, center piece longer ventrally than dorsally, expanded at both ends. Anchor pairs dissimilar, lateral anchor pair with delicate shafts and lunate ends, 0.030 (0.028-0.032) long; medial anchors longer, with delicate shafts and sickle-shaped ends, 0.041 long. Mouth subterminal. Pharynx pyriform, 0.027 long by 0.019 (0.018-0.020) wide; esophagus long, ramified laterally. Gut bifurcate, crura ramified, rami branched, crura con-

fluent posteriorly to testes. Testes follicular, 20-23 in number, roughly oval in outline, between intestinal crura postequatorially; vas deferens running anteriorly on right side. Cirrus muscular, 0.056 (0.054-0.058) long by 0.024 (0.023-0.026), eversible and protrusible with finger-like papillae in distal portion of lumen, papillae probably project outwardly when cirrus everts; rounded chamber at base of cirrus apparently functions as seminal vesicle and prostate reservoir. Numberous prostate cells around cirrus, between and lateral to intestinal crura. Genital pore ventral to gut bifurcation, opens into unarmed genital atrium. Ovary pretesticular, saccate, in a reverse J-shape, with long end dextral; oviduct running anteriorly from right ovarian lobe. Ootype fusiform, dorsal to vitello-vaginal reservoir; uterus running anteriorly in midline to enter genital atrium from right side. Genito-intestinal canal joining right crura with oviduct. Vaginal pore dextro-marginal near level of genital atrium; vaginal duct coursing obliquely to fuse with vitello-vaginal reservoir, slight muscular section placed distally on vaginal duct with folded lumen; cuticularized; hollow, cone-shaped vaginal sclerite, 0.037 (0.35-0.039) long by 0.013 (0.013-0.014) wide, anterior to vaginal pore, with a curious ramified duct at its medial end. Mehlis' gland present. Vitellaria follicular, near intestinal crura, from level posterior to vaginal pore to near posterior edge of body; transverse vitelloducts fusing medially to form Y-shaped vitello-vaginal reservoir. No eggs observed. Excretory pores supramarginal, anterior to level of genital atrium.

Discussion: *Axinoides truncatus* n. sp. is different from all other known members of the genus in the following features; (1) striking subtriangular body shape with opisthaptor obliquely situated, (2) internal organ arrangement *e.g.* junction of vaginal duct with vitello-vaginal reservoir, (3) left intestinal rami more extensive. *A. truncatus* is apparently not very closely related to the other members of the genus.

Axine resplendens Caballero, Bravo and Grocott, 1954 from *Tylosurus fodiator* is much like the present species in general body form but differs in the following characters: (1) armed genital atrium, (2) very unusual prohaptor, (3) arrangement of clamps on opisthaptor, (4) unusual clamp sclerites. *A. resplendens* evidently also possesses two pairs of anchors. This is the first notation of this feature in the genus *Axine*. The details of the clamp sclerites which are given by Caballero, Bravo and Grocott (1954) are so unusual for a member of this subfamily that they should be restudied and redescribed. The hosts of both species are closely related.

Cemocotyle carangis (MacCallum, 1913) Sproston, 1946

Synonyms: *Microcotyle carangis* MacCallum, 1913 and *Gotocotyla carangis* (MacCallum, 1913) Meserve, 1938.

Host: *Caranx crysos* (Mitchill), Blue Runner, a nerito-pelagic marine carangid.

Location: Gills.

Locality: Alligator Harbor, Florida.

Previously reported host and locality: *Caranx crysos* from North America by MacCallum (1913).

Number studied: 68.

Discussion: *Cemcotyle carangis* needs redescription. This applies particularly to the armament of the genital atrium and the clamps and anchors. It appears superficially as though *Cemcotyle* Sproston, 1946 and *Heteraxine*

Yamaguti, 1938 are very similar, particularly in the light of the probable insignificance of general clamp shape as a taxonomic criterion. Interestingly both occur on carangids. It may be that the differences here are subgeneric and not generic in value.

Genus *Heteraxine* Yamaguti, 1938

It seems probable that the genus *Heteraxine* is very closely related to the microcotylinid group. The reasons behind this assumption are: (1) cotylophore less asymmetrical than other axininids, (2) anchors apparently not persistent, (3) vagina apapillate, apparently like that of some *Microcotyle* spp. Four of the seven known *Heteraxine* spp. are parasitic on members of the piscine family Carangidae. The other three species occur on one member of each of the families Pomadasidae, Serranidae and Sciaendae.

Heteraxine carangis (MacCallum, 1918) Yamaguti, 1938.

SYNONYMS: *Axine carangis* MacCallum, 1918 and *Axine (Heteraxine) carangis* (MacCallum, 1918) Yamaguti, 1936. (Subgenus *Heteraxine* Yamaguti, 1936 given generic rank by Sproston, 1946).

HOST: *Caranx hippos* (Linn.), Common Jack, a nerito-pelagic marine carangid.

LOCATION: Gills.

LOCALITY: Alligator Harbor, Florida.

Previously reported host and locality: *Caranx hippos* from N. Y. aquarium by MacCallum (1918).

Number studied: 81.

DISCUSSION: This species needs redescription. The present host record verifies that of the original author who collected his hosts from a public aquarium.

Heteraxine obligoplitis (Meserve, 1938) Hargis 1954

SYNONYMS: *Axine oligoplitis* Meserve, 1938 and *Axinoides oligoplitis* (Meserve, 1938) Sproston, 1946.

HOST AND LOCALITY: *Oligoplites saurus* (Bloch and Schneider) from San Francisco, Equador.

DISCUSSION: Although this worm is not in the present collection, it is included for taxonomic reasons. The new combination was made by Hargis (1954) because *H. oligoplitis* is more closely related to members of the genus *Heteraxine,* with its two lateral rows of clamps, one shorter than the other, similar genital corona and apparent absence of anchors in the adult, than it is to *Axinoides* spp.

Heteraxine xanthophilis n. sp. (Figs. 10-14)

HOST: *Leiostomus xanthurus* Lacépède, Spot, a bentho-littoral marine sciaenid.

LOCATION: Gills.

LOCALITY: Alligator Harbor, Franklin Co., Florida.

Number studied: 18.

Number measured: 5.

HOLOTYPE USNM Hel. Coll. No. 38158.

DESCRIPTION: Body elongate, flattened dorso-ventrally, 2.1 (1.9-2.4) long by 0.341 (0.331-0.357) wide, sides tapered gently anteriorly, anterior end bluntly rounded, body widened posteriorly to merge inconspicuously with

cotylophore. Cuticle apparently thin and smooth. Prohaptor a pair of biloculate buccal suckers, 0.054 (0.047-0.061) in diameter, placed laterally in buccal funnel; 3 dorso-lateral head organs present on mouth rim, connected by ducts to posterior cephalic glands. Opisthaptor an asymmetrical cotylophore with laterally directed end (The direction in which end points, right or left, varies individually, but the internal organs appear to maintain a constant orientation regardless of this variance.), somewhat triangular in shape, 0.8 (0.6-0.9) wide; bearing 48-55 clamps in two unequal lateral rows, long clamp row, 29-34 clamps, on side opposite direction of end, short clamp row, 18-21 clamps, on same side as direction in which end points. Anchors apparently not persistent in adult. Clamps similar in structure, larger anteriorly than posteriorly, anterior clamps, 0.057 (0.049-0.063) long by (4) 0.040 (0.036-0.046) wide, posterior clamps 0.044 (0.039-0.051) long by 0.034 (0.030-0.036) wide, clamps microcotylid in nature, sclerites fairly delicate, ventral loop incomplete medially, but not laterally, dorsal loop elements slightly curved, middle loop incomplete medially but not laterally, center piece bifurcated at both ends. Mouth subterminal. Pharynx ovoid, 0.059 (0.049-0.063) long by 0.048 (0.046-0.054) wide; esophagus short, with two lateral rami. Gut bifurcated, crura ramified medially and laterally, rami forked, crura not confluent posteriorly, extending into haptor with left crus longer. Testes follicular, about 9-15 in number, between intestinal crura postequatorially near level of anterior clamps; vas deferens running anteriorly in midline. Short, muscular cirrus not clearly observed. Genital pore in midventral line near level of gut bifurcation, opening into armed genital atrium. Genital atrium armed by 2 laterally placed reniform muscular pieces of slightly unequal length with the left largest, (8) 0.040 (0.034-0.050) each armed medially by 2 to 4 long spines, (4) 0.016 (0.015-0.016) long, placed anteriorly and posteriorly on pads, and 4 to 7 short spines, (4) 0.008 (0.007-0.008) long, in middle of the pads. Ovary tubular, folded, pretesticular; oviduct running posteriorly from right ovarian lobe. Ootype dorsal to vitelline reservoir; uterus anterior in midline to genital atrium. Genito-intestinal canal joining right crus. Vagina not observed. Mehlis' gland present. Vitellaria follicular, near intestinal crura, extending from level of genital atrium to near posterior end of left crus in opisthaptor; transverse vitelloducts fusing medially to form Y-shaped vitelline reservoir. Eggs fusiform, (1) 0.157 long by (1) 0.069 wide, rounded at one pole, with long terminal filament at other. Brain and excretory vesicles not observed.

DISCUSSION: *Heteraxine xanthophilis* n. sp. appears to be most closely related to *H. chinensis* (Yamaguti, 1937) Yamaguti, 1938 from which it differs in the following characters: (1) nature of genital atrium armament, spines of present species in only one row, (2) transverse vitelloducts shorter, (3) more clamps on short side, (4) vitellaria less dense medially, (5) host. The host *Leiostomus xanthurus* belongs to the family Sciaenidae, a new host family for this genus.

SUMMARY

The subfamily Axininae Monticelli, 1903 has been revived and rediagnosed. The genus *Axinoides* Yamaguti, 1938 has also been emended. It has been suggested that the genera *Cemocotyle* Sproston, 1946 and *Heteraxine* Yamaguti 1938 are essentially very similar and may be congeneric.

Further work should be done to clarify this point. Hargis' (1954) recombination of *Heteraxine oligoplitis* (Meserve, 1938) has been supported.

The species *Axinoides gracilis* (Linton, 1940) Sproston, 1946, *A. raphidoma* n. sp., *A. truncatus* n. sp., *Axine resplendens* Caballero, Bravo and Grocott, 1954, *Cemocotyle carangis* (MacCallum, 1913) Sproston, 1946, *Heteraxine carangis* (MacCallum, 1918) Yamaguti, 1938 and *H. xanthrophilis* n. sp. have been described and/or discussed. Notes on host-specificity, phylogeny and other biological phenomena have been given.

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The Hermaphroditic Nature of *Thompsonia* (Crustacea: Rhizocephala) With the Description of *Thompsonia cubensis*, n. sp.

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Colonial Rhizocephala of the genus *Thompsonia* parasitize a wide variety of decapod Crustacea. The external sacs are found fastened to the abdomen, thorax or appendages of the body. They are globular, ovoid, elongate or pear-shaped structures, usually less than 3 mm. in length, and arise from a common root system. The sac consists of a mantle enclosing a visceral mass without an intervening mantle cavity. The larvae are liberated in the cypris stage.

Knowledge of the internal anatomical peculiarities of *Thompsonia* is chiefly due to the work of Coutière (1902), Häfele (1911) and Potts (1915), but the question of the animal's sexual nature is still unsettled. Is *Thompsonia* hermaphroditic like the great majority of Rhizocephala, or purely female and parthenogenetic like *Sylon,* or does it, like *Mycetomorpha,* rely on cypris males to fertilize the eggs? The answer to this question is given by the studies reported on here.

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The original description of *Thompsonia* by Kossmann, (1872) and the account of *Thylacoplethus* (the name is a synonym of *Thompsonia*) by Coutière (1902 a, b, c, d, e) make no mention of male organs. Häfele (1911) described in *Thompsonia japonica* a "complex of cells" situated near the apical pole of the animal which he interpreted as a testis. It contained cells that resembled the spermatogonia of other Rhizocephala and many were in active division. They clustered around several cells whose nuclei were four to five times as great as those of the surrounding cells. This second type of cell was of smaller size than the eggs in the same specimen and totally unlike them in structure. Häfele regarded them as "nurse cells" (Nährzellen) for the developing sperm. Since he could not find this organ in the young stage of the parasite, nor when embryos were present, he assumed it developed only at the time the eggs matured and later disintegrated as the animal grew older.

Potts (1915) denied that testes exist in *Thompsonia*. He admitted that there was an apical portion of the visceral mass where proliferation of nuclei takes place but considered this to be nothing but active embryonic tissue. The large cells with extraordinarily large nuclei he dismissed as "eggs cells rather retarded in devleopment." His verdict, which has remained unchallenged up to now, was this: "No spermatozoa are seen at any stage examined and an inspection of Häfele's figures does not inspire much confidence in his conclusion that *Thompsonia* is hermaphrodite."

Materials

The opportunity to look into the question of the disputed reproductive structures of *Thompsonia* presented itself when the senior author received for study from the Museum of Comparative Zoology at Harvard University an extensive collection of Rhizocephala occurring on various anomuran hosts. Included in the lot were eight specimens of *Munida stimpsoni* A. Milne Edwards parasitized by a species of *Thompsonia*. These had been collected by the "Atlantis" off the north coast of Cuba in 1938 and 1939.

Several representative sacs were removed from each host for measurement and examination. Twenty of these, including all available size categories, were cut into serial sections at 10 microns and stained with hematoxylin and eosin.

Ten species of *Thompsonia* are mentioned in the literature; nine of these are from the Indo-Pacific region, the other from the Bay of Naples. The presence of *Thompsonia* in a widely separated geographical region and its occurrence on a hitherto unreported host are not in themselves sufficient evidence for establishing a new species. However, in addition, this form on *Munida stimpsoni* presents a combination of characters not found in any of the named species. We therefore designate it as *Thompsonia cubensis*, new species, and include a description of it here.

Thompsonia cubensis, n. sp.

Host.—*Munida stimpsoni* A. Milne Edwards.

Material examined.—Old Bahama Channel off Punta Alegre, north coast of Cuba, 150-180 fathoms, March 11, 1938; four hosts with 6, 23, 30 and 80 parasites respectively. M. C. Z. Nos. 11478, 11479. Same locality 200-230 fathoms, April 29, 1939; two hosts with 36 and 72 parasites respectively. M. C. Z. Nos. 11483, 11484.

Old Bahama Channel off Cayo Coco, north coast of Cuba, 225 fathoms, April 27, 1939; one host with 47 parasites. M. C. Z. No. 11485. Same locality, 180 fathoms, April 28, 1939; one host with 40 parasites. M. C. Z. No. 11486.

COLLECTOR: All the specimens were collected by the Atlantis expeditions to the West Indies under the joint auspices of the University of Havana and Harvard University.

DESCRIPTION.—The parasites are attached to the underside of the abdomen, the underside of the thorax and the basal portions of the abdominal and thoracic appendages. In one case they are present also on the maxillipeds and the sides of the rostrum.

The external sacs exclusive of the stalk measure from 0.5 to 2.0 mm. in length and from 0.4 to 1.5 mm. in width. The sac is globular when small but becomes ovoid as it grows larger. Mature sacs average 1.4 mm. in length and 1.1 mm. in width. The length of the peduncle at any stage is only one-sixth to one-seventh the length of the sac. Its average length in mature specimens is 0.24 mm. Cypris larvae are present in the largest individuals, indicating that on *M. stimpsoni* this parasite probably does not exceed the maximum size given above. On some hosts the parasites vary considerably in size and degree of development, but on others they are quite uniform. A birth pore is absent.

The internal anatomy of this species is described in the following section under "Additional Observations."

REMARKS.—The species of *Thompsonia* may be separated into long-stalked and short-stalked forms. The only short-stalked forms having an ovoid or elongate shape are *T. haddoni* (Coutière) and *T. mediterranea* Caroli. But in these the shape of the sac is more slender than it is in *T. cubensis* and they are found only on the abdominal sternites of the host. Coutière describes the stalk of *haddoni* as "un court pedicule" but does not give its actual length. The stalk of *mediterranea* is one-fourth the length of the sac (Caroli, 1929).

ADDITIONAL OBSERVATIONS

The nonmuscular mantle consists of an external and internal cuticle formed respectively by an outer and inner epithelium. Cytoplasmic strands connect the two borders. The outer epithelium is continuous with the inner epithelium at the apical pole. The nuclei of the epithelia are spindle-shaped, set close together, and form a single layer at both surfaces.

Inside the mantle and in close contact with it in young specimens is a delicate tissue with rounded nuclei (innere Gewebeschicht of Häfele) that serves as an investment for the visceral mass. Later a space develops between the mantle and the visceral mass. Until the time the embryos appear this mass is made up entirely of developing eggs and interstitial tissue except for a hollow extension of the peduncle which passes partway through the center of it and undoubtedly serves to carry nourishment to the eggs. There are no oviducts or colleteric glands.

In the youngest stage examined the eggs are approximately 20 microns in diameter. In mature forms they reach a size of 80 to 95 microns.

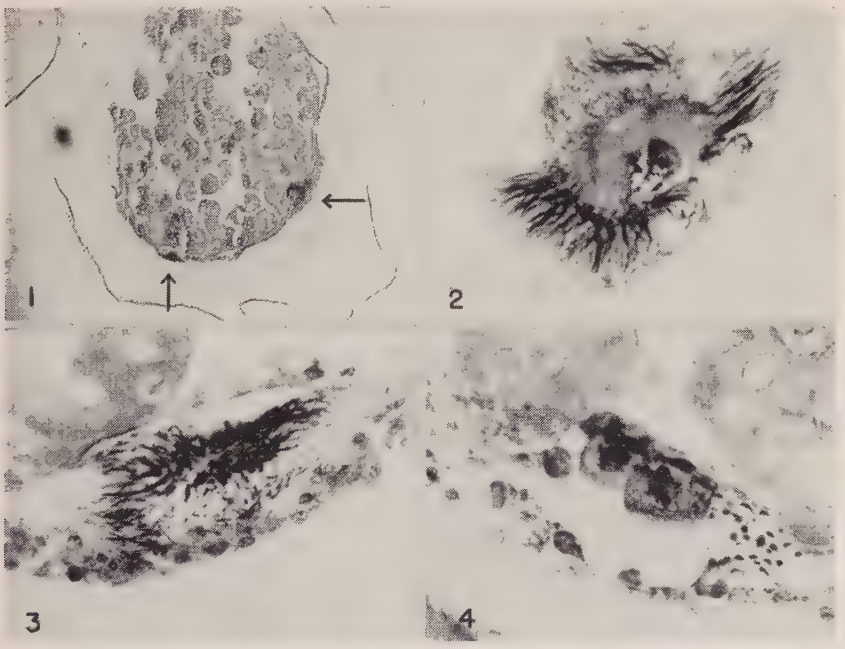
No testes were seen in the youngest animals, but in the stage where the eggs are approaching their maximum size organs have been found that are unquestionably sperm-producing. They are located, as Häfele said, a little to the side of the apical pole of the animal, but whereas he described the

male organ as a single testis, in our material they are paired, although one is smaller than the other.

The testes of *Thompsonia cubensis,* at the height of their development, are shown in figures 1 and 2. The organ then consists of two large cells, each about 30 by 16 microns, densely surrounded by filamentous sperm cells. These are arranged radially around a nurse cell with their proximal ends embedded in its cytoplasm. The nucleus of the nurse cell shows the features mentioned by Häfele: large size, irregular shape and chromatin massed at one end.

In figure 3 the testis of another animal is shown in a plane that does not include the large central cells. Here the sperm have a thicker appearance and presumably are not yet fully formed. The cells seen at the outer edge of the sperm mass with nuclei of different sizes may be interpreted as stages in spermatogenesis.

Figure 4 shows an exhausted testis. The two nurse cells remain, but spermatozoa are no longer present. The eggs of this specimen are in the stage of early cleavage, which confirms the supposition that the sperm cells have fulfilled their function. They apparently fertilize the eggs *in situ,* since embryonic development takes place within the visceral mass and not, as in other Rhizocephala, in a mantle cavity.



Figs. 1-4. The testes of *Thompsonia cubensis.* (Photomicrographs taken by Henry F. Mengoli.) 1. Longitudinal section of mature sac showing the two testes (arrows) at the edge of the visceral mass. Egg size of specimen 70 to 75 microns. X 50. 2. One testis of the same animal shown under high magnification. A nurse cell appears in the section surrounded by sperm cells. X 450. 3. A younger testis with sperm incompletely developed. The section does not include a nurse cell. Egg size of specimen 60 to 65 microns. X 450. 4. A testis after the release of the sperm. Two nurse cells may be seen near the center of the photograph. Egg size of specimen 75 to 85 microns. X 450.

Fig. 5. Longitudinal section of a young sac of *Thompsonia cubensis*. Egg size of specimen 25 microns. The apical pole lies at the right of the figure. X 200.

With the progress of larval development the visceral mass disintegrates. None of the sectioned animals containing advanced embryos or cypris larvae showed any remnants of testes.

These observations prove that functional testes appear in *Thompsonia* at the time the animal's eggs are ready for fertilization, but once the sperm are liberated the male organs disappear. It now becomes necessary to explain how and when the sperm-producing bodies originate.

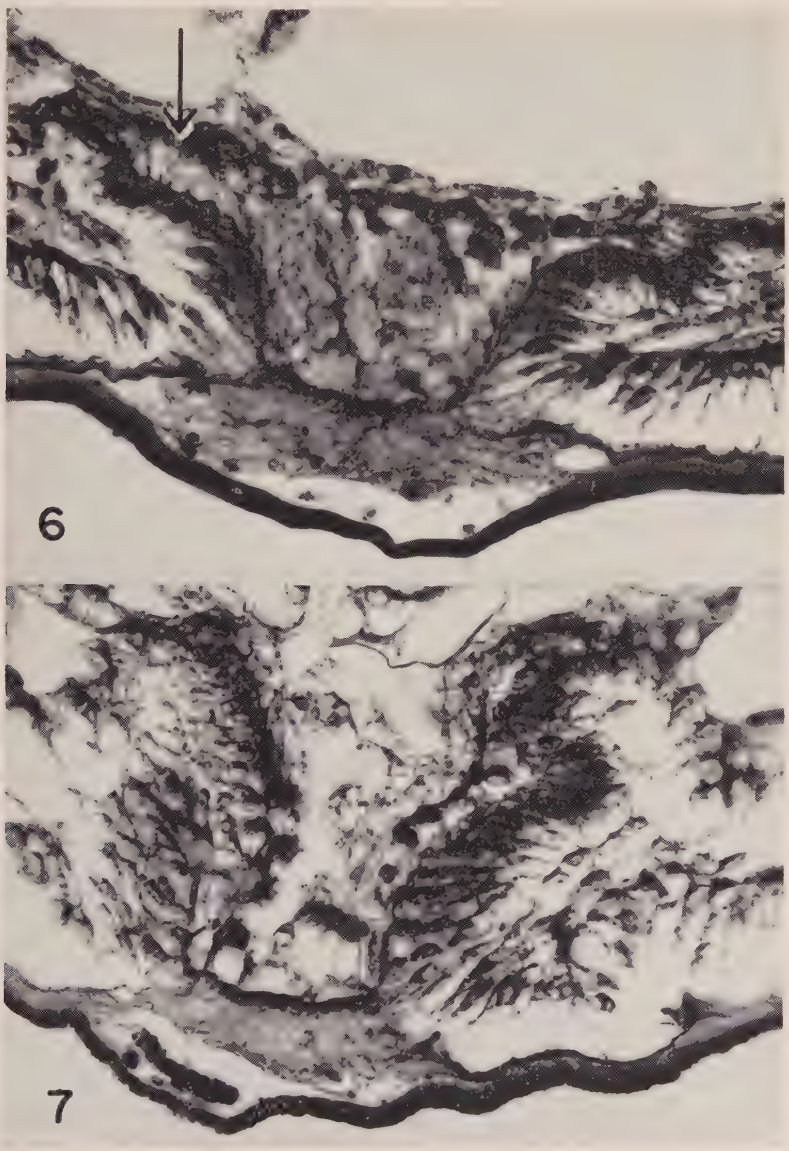
The development of testes is associated with the proliferation of a cap of high cells at the apical pole (Fig. 5.) These cells belong to the investing layer. They penetrate a cul-de-sac of the mantle formed by reflexion of the exterior mantle epithelium which at this point becomes the inner mantle epithelium. Here the cap forms a somewhat cone-shaped intrusion (Fig. 6.) Immediately surrounding it the mantle shows a definite zone of thickening (Fig. 7.)

This cap was seen in eight of the sectioned animals. It appeared most clearly in specimens with eggs of about 25 to 50 microns in diameter.

The primordia of the testes make their appearance in the out-spread basal margins of the cap which extend beneath the thickened zone of the mantle. The distance separating them at first is less than 100 microns. But as the eggs increase in size, expansion of the ovary causes a stretching of the investing layer and the mantle. Both of these become greatly reduced in thickness. The cap disappears and the testes, now much farther apart and fully developed, lie embedded in the surface of the ovarian mass. In the specimen photographed (Fig. 1) the distance between the two testes in the same plane is about 300 microns.

DISCUSSION

Coutière (1902 c, e) mentions that in young specimens of *Thylacoplethus* the distal portion of the visceral mass produces a cap of high cells (*calotte terminale*) which penetrates a circular depression in the inner layer of the



Figs. 6-7. Details of apical pole seen in young specimens of *Thompsonia cubensis*. (Photomicrographs taken by Henry F. Mengoli.) 6. The terminal cap protruding into the mantle at the point where the outer epithelium is reflexed. Arrow points to site of origin of testis. X 900. 7. A terminal cap with the thickened zone of the mantle surrounding it. X 900.

mantle. This he interpreted as the beginning of a "cloacal aperture" which the next moult would uncover. In the young stages sectioned by us the same type of configuration was observed, but no sac, small or large, developed an apical aperture. The *calotte terminale,* in our material, seems to be primarily concerned with the development of the testes, rather than with the formation of a birth pore.

Potts (1915) makes no mention of a terminal cap growing upwards from the visceral mass. Moreover, he saw no sign of any organ that in his opinion could be called a testis. Perhaps the sacs he sectioned were too young or too old to show definitive male organs. Obviously, the present work invalidates his conclusion that *Thompsonia* is parthenogenetic.

Häfele's (1911) opinion that *Thompsonia japonica* is hermaphroditic in nature is substantiated by the conclusions arrived at here with respect to *Thompsonia cubensis.* The chief difference is that the former is reported to have a single testis, while the latter has paired male organs. In both cases the testes disappear after larval development begins.

Summary

A new species of Rhizocephala occurring on *Munida stimpsoni* A. Milne Edwards from the north coast of Cuba is described under the name *Thompsonia cubensis.*

In this species reproduction is not parthenogentic but hermaphroditic. The testes originate alongside the apical pole of the animal in connection with the proliferation of a "terminal cap" derived from the investing layer of the visceral mass. Later they come to lie embedded in the surface of the ovary where they reach their full development at the time the eggs are ready for fertilization. Each testis then consists of a small cavity containing two nurse cells densely surrounded by spermatoza. The eggs are apparently fertilized *in situ.* Subsequently the testes disintegrate.

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Constitutional Amendment

Approved, 339th Meeting, April 20, 1956

Article 3, Section 4, last sentence: " . . . shall not exceed three at any one time"; is changed to " . . . shall not exceed FIVE PERCENT OF THE RESIDENT AND NON-RESIDENT MEMBERSHIP AT THE TIME OF ELECTION."

MINUTES

Three Hundred Thirty-third to Three Hundred Thirty-ninth Meetings

333rd. meeting: McMahon Hall, Catholic University of America, Washington, D. C., October 19, 1955. Twenty dollars voted to help defray expenses of Picnic held in June. Papers presented: *Sarcocystis* from raccoons, by Herman and Habermann; *Eurytrema procyonis* from raccoons and foxes in Maryland, by Herman and Bauman; The quality of carbohydrate and its effect on *Hymenolepis* in the rat, by Read; A study of the anthelmintic activity of Maklua berry (*Diospyros mollis*) and other persimmons, by Luttermoser; Effect of cadmium compounds as anthelmintics in treatment of *Ascaris* of swine, by Harwood; History of the development of our knowledge on the life cycle of *Fasciola hepatica,* by Reinhard.

334th. meeting: Log Lodge, Agricultural Research Center, Beltsville, Md., November 25, 1955. Voted J. Andrews representative to Washington Academy of Science. Papers presented: Some new materials used for culture of nematode larvae, by Shorb; Observations on the devlopmental stages of the cysticercoid of the fowl tapeworm, *Raillietina cesticillus* (Molin), by J. C. Hwang; Eosinophilia in bovine lungworm disease, by Rubin; Nicarbazin as a coccidiostatic drug in *E. tenella* infections in chickens, by McLoughlin; Some observations on scabies of sheep and cattle, by Peterson.

335th. meeting: Sternberg Auditorium, Walter Reed Army Institute of Research, Washington, D. C., December 14, 1955. Officers elected for 1956: F. D. Enzie, President; D. McMullen, Vice President; E. Buhrer, Corresponding Secretary-Treasurer; G. Anastos, Recording Secretary. Papers presented: A review of research on filariasis in Malaya, by Jachowski; Notes on animal filarial parasites in Malaya with special reference to *Macaca irus,* by D. L. Price and Jachowski; Parasitological studies in Alaska, by Wallace; Some properties of an encephalitozoon-like organism isolated from mice, by Morris; The problems of control and therapy of anaplasmosis in Jamaica, B. W. I., by V. M. Young; The "nasal-leech," *Dinobdella ferox,* from Malaya, by Walton.

336th. meeting: McMahon Hall, Catholic University of America, Washington, D. C., January 18, 1956. Twelve dollars voted for Science Fair of the Washington Academy of Science. Voted L. Oliver as member of Executive Committee. Papers presented: The effect of administration of a nonspecific antigen on the development of acquired immunity to *Nippostrongylus muris* in the rat, by O'Keefe; Blood cell changes in normal and immune rats during infection with *Nippostrongylus muris,* by Zam; A *Trypanosoma cruzi*-like Trypanosome from the raccoon, by Walton, Bauman, Diamond and Herman; An intracellular parasite in the red blood cell of the raccoon, by

Diamond; Report of the finding of *Strongylus asini* in the zebra, by Colglazier.

337th. meeting: Silvester Hall, University of Maryland, College Park, Md., February 15, 1956. Papers presented: Distribution and variation of *Pratylenchus* species in Maryland, by Taylor; Host-parasite relationships between *Retylenchus buxophilus,* n. sp., and boxwood, by A. M. Golden; Morphogenesis of the parasitic stages of *Ostertagia ostertagi,* a nematode parasite in cattle, by Douvres; Some parasites of the English sparrow in Maryland by Wilson; On the occurrence of *Paragoniums* sp. in swine in Georgia, by T. B. Stewart; Development of *Hepatozoon sciuris* Coles, 1914 in two parasitic laelaptid mites, by Clark.

338th. meeting: National Institutes of Health, Bethesda, Md., March 23, 1956. Executive Committee recommended that $70.55 be transferred from general fund to publication fund; that Treasurer deposit $500.00 from matured Series G. Bond in Hyattsville Building Association if covered by adequate insurance; that manuscripts be accepted for the Journal without additional charge unless papers are inordinately long, have excessive tabulation or illustrations. Voted to accept financial report of Treasurer. Papers presented: The *in vitro* cultivation of *Nippostrongylus muris* to the adult stage, by Weinstein and Jones; The occurrence of a dog filariid other than *Dirofilaria immitis* in the United States, by Newton; The epidemiology of human trichomoniasis, by Burch.

339th. meeting: Johns Hopkins University School of Hygiene and Public Health, Baltimore, Md., April 20, 1956. Voted to amend last sentence of Article 3, Section 4, of the Constitution to read "The number of life members shall not exceed 5% of the resident and non-resident membership at the time of election." Voted to elect Dr. Christie and Dr. Steiner to life membership. Voted twenty dollars to defray expenses of picnic in May. Papers presented: Host specificity of the rat nematodes, *Nippostrongylus muris,* by Haley; Reactivation phenomenon in Rocky Mountain Spotted Fever, by Gilford; The inheritance of autogeny in *Culex pipiens* mosquitos, by Spielman; Carbohydrate metabolism of *Moniliformis, Hymenolepis diminuta* and *Oochoristica symmetrica,* by Laurie; The role of the skin in the development of *Nippostrongylus muris* in the rat, by Twohy.

The following were elected to membership during the year: 333rd meeting—Alvin J. Braun, Richard A. Rohde, Charles F. Needy, Cornell A. Johnson, George T. Riggin, Jr., Warren R. Pistey, Thomas W. Mabbott, Paul H. Allen, John G. Atkins, Norman D. Levine, Stanley T. Ichikawa, Robert S. Isenstein, George H. Rohrbacher, Jr., Chancellor I. Hannon; 336th.—Bradner W. Coursen, C. Robert Peebles, Arwed H. Meyl; 338th.—Bruce E. Hopper, Norman A. Minton, Walter J. Apt, R. P. Esser, Eugene B. Sledge, Robert M. Stabler, Wray Birchfield, Georgiana L. Brown, T. T. Dunagan; 339th.—Robert L. Bray, John I. Bruce, Jr., Tibor Boros, A. D. Baker, A. J. Overman, Alvin H. Rothman, T. A. Hymas, Edward H. Wilkens.

George Anastos
Recording Secretary

IN MEMORIAM

Sydney Eliott Askinas

August 28, 1927 — February 19, 1956

Graduate Student in the Department of Zoology,
University of Maryland
College Park, Maryland

Member of Helminthological Society of Washington Since November, 1954

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